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Caffeinated and Decaffeinated Coffee Consumption and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis

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ABSTRACT

Objectives: To examine the association between caffeinated and decaffeinated coffee with hepatocellular carcinoma (HCC) including the influence of HCC aetiology and pre-existing liver disease.

Design: We performed a systematic review and meta-analysis. We calculated relative risks (RRs) of HCC according to caffeinated and decaffeinated coffee consumption using a random-effects dose-response meta-analysis. We tested for modification of the effect estimate by HCC aetiology and pre-existing liver disease.

Results: We found 18 cohorts, involving 2,272,642 participants and 2,905 cases, and 8 case-control studies, involving 1,825 cases and 4,652 controls. An extra two cups/day of coffee was associated with a 35% reduction in the risk of HCC (RR 0.65, 95% CI 0.59-0.72). The inverse association was weaker for cohorts (RR 0.71, 95% CI 0.65-0.77), which were generally of higher quality than case-control studies (RR 0.53, 95% CI 0.41-0.69). There was evidence that the association was not significantly altered by stage of liver disease or the presence/absence of high alcohol consumption, high body mass index, type-2 diabetes mellitus or hepatitis B and C viruses. An extra two cups of caffeinated and decaffeinated coffee (2 and 3 cohort studies, respectively) were associated with reductions of 27% (RR 0.73, 95% CI 0.63-0.85) and 14% (RR 0.86, 95% CI 0.74-1.00) in the risk of HCC.

Conclusions: Increased consumption of both caffeinated and decaffeinated coffee is associated with reduced risk of HCC, including in pre-existing liver disease. These findings are important given the increasing incidence of HCC globally and its poor prognosis.

ARTICLE SUMMARY

Strengths and limitations of this study

Strengths:

- This is the first meta-analysis to calculate clinically relevant RRs of HCC for 1-5 cups of coffee per day.
- This is the first meta-analysis to investigate the influence of all the main HCC risk factors on the association between coffee and HCC.
- This is the first meta-analysis to calculate a RR of HCC for decaffeinated coffee consumption.

Limitations:

- There was heterogeneity between the studies included in the meta-analysis.
- Many studies did not specify coffee caffeine content.

INTRODUCTION

Primary liver cancer is the sixth most commonly diagnosed cancer worldwide and, because of its poor prognosis, the second leading cause of cancer death.[1, 2] Hepatocellular carcinoma (HCC) is the dominant histological subtype accounting for 85-90% of cases.[3] HCC most commonly develops in people with cirrhosis due to chronic viral hepatitis B (HBV) or C (HCV), excess alcohol consumption and/or non-alcoholic fatty liver disease (NAFLD).[3] Non-alcoholic steatohepatitis (NASH), which is rapidly increasing worldwide, can lead to the development of HCC in the absence of cirrhosis.[4] The incidence of liver cancer is increasing due to changes in these underlying risks and by 2030 the number of new cases annually will have risen by around 50% to over 1.2 million.[5] The burden of liver cancer is highest in East and South East Asia, with China alone accounting for 50% of cases worldwide.[2] Only a minority of patients present at a stage where they are eligible for potentially curative interventions (such as liver transplantation or partial liver resection), and the availability of such treatments is limited in areas most affected by HCC. Prognosis remains poor with a 5-year overall survival rate of 18%.[6]

Coffee is a popular drink in most countries with approximately 2.25 billion cups consumed daily.[7] It is a complex mixture of biologically active molecules, including caffeine, chlorogenic acid and diterpenes.[8] These compounds possess anti-oxidant, anti-inflammatory anti-fibrotic and anti-carcinogenic properties which may explain observational data that coffee drinkers have lower rates of chronic liver disease (CLD), including fibrosis, cirrhosis and HCC.[9] A recent meta-analysis reported that the relative risk (RR) of HCC for an extra cup of coffee per day was 0.74 (95% CI 0.65-0.83).[10] We have now explored, for the first time in a meta-analysis, the modification of the inverse association between coffee and HCC by key risk factors, such as HBV/HCV, high body mass index (BMI), type-2 diabetes mellitus (T2DM), alcohol consumption and the presence of CLD including cirrhosis.

We also report the first meta-analysis for the association between decaffeinated coffee and HCC. Decaffeinated coffee protects against liver damage in animal studies[11] and is inversely associated with T2DM, abnormal liver function tests (LFTs) and cirrhosis in human observational studies.[12-14]

METHODS

The methods used were similar to those described in our earlier work,[15] and are detailed below. We followed the PRISMA guidelines; a protocol is provided as supplementary information.

Searches and selection of studies

We performed searches of abstracts and titles in Web of Science, Embase, and PubMed with: (“odds” OR “risk” OR “hazard” OR "OR" OR "RR" OR "HR") AND “coffee” AND (“liver” OR “hepatocellular*”) AND (“cancer“ OR “carcino*” OR “neoplas*”). The searches were run in September 2015 without restriction of date of publication. References of pertinent studies were searched manually. After removing duplicates, OJK and RB independently screened the titles and abstracts of the studies found in the search. Studies were included that: (i) reported a RCT, case-control study or cohort study; and (ii) reported hazard ratios (HRs), odds ratios (ORs) or relative risks (RRs) with 95% confidence intervals (95% CIs) for HCC in adults according to consumption of coffee. Studies were excluded that i) did not report a dose-response or give sufficient information for calculation of a dose-response (i.e. this requires estimates for more than two exposure levels, or ii) were in a non-English language. We assumed cases of primary liver cancer to be HCC. If studies overlapped, we included the largest study or otherwise the last published study. We worked from published studies only, including abstracts.

Extraction of data and assessment of quality

We extracted the following information from each study: the first author, the date of publication, the geographic region, the design of the study, the exclusion and inclusion criteria, the estimates and adjustments, the numbers of participants (or controls) and cases, the methods of measuring exposure and case identification. We also extracted data concerning cohort follow-up (time, losses) and whether baseline liver disease was excluded. We extracted the most rigorously adjusted effect sizes. We extracted effect sizes stratified by pre-existing CLD, alcohol consumption, BMI, hepatitis B and C virus status, T2DM, and type of coffee. OJK extracted the data which RB then checked. Given the low incidence of HCC, we considered ORs, RRs, HRs to be equivalent, and for simplicity we use RR to refer to all three herein. We assessed the quality of the included studies using the Newcastle-Ottawa Scale.[16] We judged the quality of evidence with Grading of Recommendations Assessment, Development and Evaluation (GRADE).[17]

Statistical methods

Coffee and HCC

Most studies did not distinguish caffeinated vs. decaffeinated coffee, so coffee was taken to be the pattern of use prevalent in the particular study population. We considered consumption in cups, where necessary[18] converting millilitres into cups of 150 mL. We estimated for each study a RR of HCC for an extra two cups per day using the method of Greenland and Longnecker.[19] We estimated median consumption for each reported consumption category to be the midpoint of closed ranges and the midpoint added to the amplitude of the previous range for open ranges.[20] We assessed whether the dose-response was non-linear by a cubic spline meta-analysis.[21] We tested for statistical heterogeneity using I^2 and Cochran's Q,[22] and interpreted p-values of <0.1 as statistically significant (for heterogeneity only) and we interpreted the I^2 values according to the Cochrane handbook.[22] We investigated

heterogeneity by meta-regression and examined the impact of individual studies by re-running the analysis while leaving the studies out one at a time.[23] We tested for publication bias using Egger's test and a "trim-and-fill" analysis,[24] which we used to adjust the estimate for missing studies if publication bias was indicated. To assess the magnitude and direction of adjustment, we calculated a pooled unadjusted effect sizes for comparison with the corresponding adjusted effect size. We used random effects models and a two sided p-value of >0.05 for statistical significance. We used R (R Foundation for Statistical Computing, Vienna, Austria) with the metafor and dosresmeta packages for the analyses.

We calculated RRs of HCC according to coffee consumption in participants stratified by baseline CLD. We also calculated and meta-analysed RRs stratified by exposure to each of: viral hepatitis status (carriers of HBV/HCV vs. negative for both), BMI (highest vs. lowest BMI categories), T2DM (presence vs. absence), or alcohol consumption (highest vs. lowest categories). For these analyses, we only included studies that provided RRs for both exposed and non-exposed to the risk factors. Where possible, we estimated the dose-response using the Greenland and Longnecker method.[19] Where the number of exposed and non-exposed were not available to correct for covariance, we used variance-weighted least squares regression. We meta-analysed the differences between the stratified RRs to test for statistical significance.

Caffeinated and decaffeinated coffee and HCC

Where possible we extracted data separately for caffeinated and decaffeinated coffee and calculated pooled RRs of HCC per two extra cups/day of each. One study, Bamia et al.,[18] reported RRs of HCC according to decaffeinated coffee consumption for three qualitative categories: "non-consumers", "consumers below the median" and "consumers at/above the median". We were unable to get the corresponding quantitative values from the authors so

used those reported by another publication investigating the effect of decaffeinated coffee on oesophageal cancer in the same cohort.[25]

RESULTS

Coffee consumption and HCC

Figure 1 shows the searches and the stages of the selection of studies. Once duplicates were removed, we screened the abstracts and titles of 181 studies. Of those, we reviewed 34 studies in their entirety. Table 1 summarises the characteristics of the 16 studies which we included in the main meta-analysis.[12, 18, 26-39] The studies were published between 2002 and 2015. Seven were from Europe, five from Japan, two from the US and one from each of Hong Kong and Singapore. The cohort studies primarily involved general populations (e.g. randomly selected from population registries) except for Lai et al.,[31] which included male smokers only. Total follow-ups ranged from seven[28] to 24 years,[31] and linkage to cancer registries was generally used to identify cases and exclude baseline HCC. The case-control studies were hospital based, with only one[38] using community controls. Fifteen studies reported estimates according to "coffee" consumption, while two and four studies, respectively, reported estimates specifically for caffeinated and decaffeinated coffee. The quality scores ranged from 4 to 8 (table 1) and were generally higher for cohorts (mean=6.9) compared to case-control studies (mean=5.0). A number of studies reported data from multiple cohorts or case-control studies. We extracted pooled estimates from Petrick et al.[32] (nine cohorts) and Gallus et al.[33] (two case-control studies) as equivalent study-specific estimates (e.g. in terms of adjustments for confounders and categories of coffee consumption) were not available. We extracted separate RRs from Shimazu et al[28] (two cohorts). Thus, this meta-analysis included data from 18 cohorts, involving 2,272,642

participants and 2,905 cases, and 8 case-control studies, involving 1,825 cases and 4,652 controls.

Table 1a. Details of the cohort studies meeting the inclusion criteria

Cohort study	Country	Population characteristics (age)	Cohort (% men)	Baseline exposure ascertainment	Outcome	Outcome ascertainment	Follow-up years	Cases (rate/1000)	NOS quality score
Inoue et al. 2005[26]	Japan	Gen pop (40-69)*	90,452 (48)	FFQ	HCC	Cancer registry, death records, medical records	9.7 (average)	334 (3.7)	7
Kurozawa et al. 2005[27]	Japan	Gen pop (40 to 79)*. HCC deaths within 1st 2 years excluded	110,688 (42)	FFQ	HCC death	Death records	9-11 (total)	258 (2.3)	7
Shimazu et al. (cohort 1) 2005[28]	Japan	Gen pop (≥40)*	22,404 (47)	FFQ	PLC	Cancer registry, death records, medical records	9 (total)	70 (3.1)	6
Shimazu et al. (cohort 2) 2005[28]	Japan	Gen pop (40-64)*	38,703 (49)	FFQ	PLC	Cancer registry, death records, medical records	7 (total)	47 (1.2)	6
Hu et al. 2008[29]	Finland	Gen pop (25-74)*	60,323 (49)	FFQ	PLC	Cancer registry	19.3 (median)	128 (2.1)	8
Johnson et al. 2011[30]	Singapore	Gen pop (45 to 74)*	61,321 (44)	FFQ	HCC	Cancer registry and death records	n/a	362 (5.9)	8
Lai et al. 2013[31]	Finland	Male smokers (50-69) from an RCT into lung cancer*. Self-reported cirrhosis excluded at baseline	27,037 (100)	FFQ	LC	Cancer registry	18.2 (median)	194 (7.2)	6
Bamia et al. 2014[18]	Europe **	Gen pop (25 to 70)*	486,799 (30)	FFQ	HCC	Cancer registry, death records, health insurance records and mail/telephone	11 (median)	201 (0.4)	7
Setiawan et al. 2015[12]	USA	Gen pop (45 to 75)*	162,022 (47)	FFQ	HCC	Cancer registry	18 (median)	451 (2.8)	7
Petrick et al. 2015[32]	USA	Gen pop (<50-≥70)*	1,212,893 (41)	FFQ	HCC	Cancer registry, medical records, self-reporting	Variable	860 (0.7)	6

Abbreviations: hepatocellular carcinoma (HCC), international classification of diseases (ICD), primary liver cancer (PLC), liver cancer (LC), intrahepatic cholangiocarcinoma (ICC), hepatitis B virus (HBV), hepatitis C virus (HCV), Newcastle-Ottawa Scale (NOS). * Participants with a diagnosis of HCC were excluded at baseline; ** Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom.

Table 1b. Details of the case-control studies meeting the inclusion criteria

Case-control study	Country	Case selection	N (% men) and age of cases	Control selection	N (%men) and age of controls	Measurement of coffee consumption	Outcome	NOS quality score
Gallus et al. 2002 (study 1)[33]	Italy	Hospital	501 (75) aged 20-75 (median 60)	Patients with non-cancer disorders in same hospital and from same catchment area	1552 (74) aged 18-75 (median 56)	FFQ	HCC	5
Gallus et al. 2002 (study 2)[33]	Greece	Hospital	333 (85) aged 31–79 (median 65)	Patients with non-cancer disorders in same hospital	360 (83) aged 24–79 (median 65)	FFQ	HCC	5
Gelatti et al. 2005[34]	Italy	Hospital	250 (82) aged less than 80 (mean 63.8)	Patients without liver disease in same hospital	500 aged less than 80 (mean 64.1)	FFQ	HCC	7
Ohfuji et al. 2006[35]	Japan	Attending hospital for HCV follow-up	73 (47) mean age 68.9	Attending hospital for HCV follow-up	253 (52) mean age 68.3	FFQ	HCC	5
Tanaka et al. 2007[38]	Japan	Hospital	209 (68) aged 40-79 (mean 67)	Community controls randomly selected	1308 (50) (mean 57)	FFQ	HCC	4
Montella et al. 2007[36]	Italy	Hospital	185 (81) aged 43-84 (median 66)	Patients in same hospital	412 (68) aged 40-82 (median 65)	FFQ	HCC	5
Leung et al. 2011[39]	Hong Kong	Attending hospital for HBV follow-up	109 (79) aged ≤39 to ≥60	Attending hospital for HBV follow-up	125 (82) aged ≤39 to ≥60	FFQ	HCC	5
Stucker et al. 2006[37]	France	Hospital	165 (100) aged <75	Patients without liver disease in same hospital	142 (100) aged <75	FFQ	HCC	4

The RRs of HCC according to coffee consumption are summarised in table 2, including adjustments for confounders. Most studies adjusted for age, alcohol and smoking, and a smaller number for HBV/HCV, BMI and T2DM. All the studies showed an inverse association between HCC for an extra two cups of coffee per day, although in four studies the relationship was not statistically significant. The pooled RR of HCC for an extra 2 cups/day across all studies for coffee was 0.65 (95% CI 0.59-0.72) (figure 2), for cohort studies it was 0.71 (95% CI 0.65-0.77) and for case-control studies 0.53 (95% CI 0.41-0.69). The pooled RR from studies with a quality score of 6 or above was 0.70 (95% CI 0.64-0.76) compared to 0.50 (95% CI 0.35-0.70) for those scoring below 6. The p-value for non-linearity of the dose-response was not statistically

significant, and the pooled RRs for different levels of consumption of up to 5 cups per day are illustrated in figure 3. Adjustment for confounders had minimal effect, changing the pooled RR from 0.62 (95% CI 0.53-0.72) to 0.65 (95% CI 0.59-0.72).

Table 2. The associations reported by the studies meeting the inclusion criteria for the main coffee-HCC meta-analysis.

Study	Coffee (cups per day, unless specified)	Participants	Cases (cumulative rate/1000)	Adjusted RR (95% CI)	Adjustments
Cohort studies					
Inoue et al. 2005[26]	Almost never 1-2/wk 3-4/wk 1-2 3-4 ≥5	29,423 17,159 10,316 23,753 7,316 2,485	161 (5.5) 65 (3.8) 36 (3.5) 54 (2.3) 15 (2.1) 3 (1.2)	1 (ref.) * 0.75 (0.56-1.01) * 0.79 (0.55-1.14) * 0.52 (0.38-0.73) * 0.48 (0.28-0.83) * 0.24 (0.08-0.77) *	Age, gender, alcohol, smoking, green tea, study area, green vegetable intake.
Kurozawa et al. 2005[27]	Non-drinkers <1 ≥1	24,556 15,259 44,151	103 (4.2) 57 (3.7) 98 (2.2)	1 (ref.) * 0.83 (0.54-1.25) * 0.5 (0.31-0.79) *	Age, gender, alcohol, smoking, T2DM, liver disease, education.
Shimazu et al. (cohort 1) 2005[28]	Never Occasionally ≥1	4,938 9,507 7,959	29 (5.9) 25 (2.6) 16 (2.0)	1 (ref.) ** 0.56 (0.33-0.97) ** 0.53 (0.28-1.00) **	Age, gender, alcohol, smoking, liver disease.
Shimazu et al. (cohort 2) 2005[28]	Never Occasionally ≥1	6,954 14,130 17,619	12 (1.7) 21 (1.5) 14 (0.8)	1 (ref.) ** 1.05 (0.52-2.16) ** 0.68 (0.31-1.51) **	Age, gender, alcohol, smoking, liver disease.
Hu et al. 2008[29]	0 to 1 2 to 3 4 to 5 6 to 7 ≥8	6,150 12,681 17,991 13,726 9,775	20 (3.3) 30 (2.4) 33 (1.8) 28 (2.0) 17 (1.7)	1 (ref.) * 0.66 (0.37-1.16) * 0.44 (0.25-0.77) * 0.38 (0.21-0.69) * 0.32 (0.16-0.62) *	Age, gender, alcohol, smoking, T2DM, liver disease, education, BMI, study year.
Johnson et al. 2011[30]	Non-drinkers 0-<1 1-<2 2-<3 ≥3	119,973 (PY) 70,762 (PY) 236,215 (PY) 190,567 (PY) 37,505 (PY)	69 38 149 92 14	1 (ref.) * 0.94 (0.63-1.40) * 1.17 (0.87-1.56) * 0.78 (0.56-1.07) * 0.56 (0.31-1.00) *	Age, gender, alcohol, smoking, T2DM, education, BMI, dialect group, year of recruitment, black and green tea.
Lai et al. 2013[31]	Never drinkers	667	9 (13.5)	1.35 (0.65-2.82) **	Age, alcohol, smoking, T2DM, education, BMI, tea,

	>0 to <1 1 to <2 2 to <3 3 to <4 ≥4 per extra cup	3,094 7,204 8,086 4,515 3,471	36 (11.6) 60 (8.3) 47 (5.8) 22 (4.9) 20 (5.8)	1 (ref.) ** 0.73 (0.48-1.12) ** 0.52 (0.33-0.82) ** 0.45 (0.26-0.78) ** 0.53 (0.30-0.95) ** 0.82 (0.73-0.93) **	cholesterol, marital status, ATBC intervention arm ¶.
Bamia et al. 2014[18]	Quintile 1 Quintile 2 Quintile 3 Quintile 4 Quintile 5	98,148 100,953 95,231 96,413 96,054	47 (0.5) 49 (0.5) 38 (0.4) 36 (0.4) 31 (0.3)	1 (ref.) * 0.85 (0.56-1.29) * 0.63 (0.39-1.02) * 0.49 (0.29-0.82) * 0.28 (0.16-0.5) *	Stratified for age and centre. Adjusted for gender, alcohol, smoking, T2DM, education, BMI, physical activity, energy intake, tea.
Setiawan et al. 2015[12]	Never <1 1 2 to 3 ≥4	44,438 31,056 45,717 32,593 8,218	119 (2.7) 111 (3.6) 137 (3.0) 67 (2.1) 17 (2.1)	1 (ref.) * 1.14 (0.88-1.48) * 0.87 (0.67-1.11) * 0.62 (0.46-0.84) * 0.59 (0.35-0.99) *	Age, gender, alcohol, smoking, T2DM, education, BMI, race.
Petrick et al. 2015[32]	Non-drinker >0 to <1 1 to <2 2 to 3 >3 per extra cup	172,950 164,977 179,781 370,786 161,116	85 (0.5) 138 (0.8) 149 (0.8) 255 (0.7) 97 (0.6)	1 (ref.) * 1.24 (0.94-1.64) * 1.16 (0.88-1.52) * 0.89 (0.68-1.15) * 0.73 (0.53-0.99) * 0.90 (0.85-0.94) *	Age, gender, alcohol, smoking, BMI, race, cohort.
Case-control studies		**Cases**	**Controls**		
Gallus et al. 2002 (Italian and Greek studies combined)[33]	Non drinkers 1 2 ≥3	129 231 292 178	256 432 582 637	1 (ref.) *** 1.2 (0.9-1.6) *** 1.0 (0.7-1.3) *** 0.7 (0.5-1.0) ***	Age, gender, alcohol, smoking, education, BMI, T2DM, hepatitis, study.
Gelatti et al. 2005[34]	No consumption 1 to 2 3 to 4 ≥5	44 119 69 18	59 206 163 72	1 (ref.) *** 0.8 (0.4-1.3) *** 0.4 (0.2-0.8) *** 0.3 (0.1-0.7) ***	Age, gender, alcohol, HBV, HCV.
Ohfuji et al. 2006[35]	Non drinkers <1 ≥1	25 19 29	63 74 116	1 (ref.) *** 0.61 (0.18-2.03) *** 0.38 (0.13-1.12) ***	Alcohol, smoking, BMI, duration of liver disease, disease severity, family history, interferon therapy, other caffeine containing beverage
Tanaka et al. 2007[38]	None Occasional 1 to 2 ≥3	127 53 17 12	268 496 268 221	1 (ref.) *** 0.33 (0.22-0.48) *** 0.27 (0.15-0.48) *** 0.22 (0.11-0.43) ***	Age, gender, alcohol, smoking, HBV, HCV.
Montella et al. 2007[36]	Abstainers <14/wk 14 to 20 21 to 27	27 67 50 27	41 116 104 88	2.28 (0.99-5.24) *** 1 (ref.) *** 0.54 (0.27-1.07) *** 0.57 (0.25-1.32) ***	Age, gender, alcohol, smoking, education, centre, HBV, HCV.

	≥28	14	63	0.43 (0.16-1.13) ***	
Leung et al. 2011[39]	No coffee habit 1-3/wk ≥4 wk	86 11 12	82 17 26	1 (ref.) *** 0.58 (0.24-1.36) *** 0.41 (0.19-0.89) ***	Age, gender, alcohol, smoking, tea, physical activity
Stucker et al. 2006[37]	0-1 2 >2	92 45 28	57 37 48	1 (ref.) *** 0.67 (0.3-1.3) *** 0.36 (0.2-0.7) ***	Alcohol

Abbreviations: person years (PY), hepatitis B virus (HBV), hepatitis C virus (HCV), α-tocopherol or β-carotene (ATBC); * reported as HR; ** Reported as RR; *** Reported as OR. ¶ Participants were from another trial investigating vitamin E supplementation in the form of α-tocopherol or β-carotene;

Heterogeneity and sensitivity analysis

I^2 and the p-value for Cochran's Q were 58.5% and <0.01 respectively (figure 2), which indicated "moderate" to "substantial" between-study heterogeneity. Heterogeneity was lower for cohorts (I^2=40.7%; p=0.09) than case-control studies (I^2= 64.3%; p<0.01). In the sensitivity analysis, the RR was strongest when we excluded Hu et al.[29] (RR 0.63, 95% CI 0.56-0.71) and weakest when we excluded Tanaka et al.[38] (RR 0.68, 95% CI 0.62-0.74). Heterogeneity remained statistically significant throughout. In the meta-regression analysis, we found no statistically significant association of RR and publication year, length of follow-up (cohorts only), percentage of alcohol abstainers, age or gender.

Publication bias and quality of evidence

We found evidence of publication bias by Eggers test (p<0.0001) and visual inspection of the funnel plot as shown in figure 4. In our trim-and-fill analysis, we detected a number of "missing" smaller studies. Calibration for missing studies pushed the effect size of coffee towards null from 0.65 (95% CI 0.59-0.72) to 0.71 (95% CI 0.64-0.79). The evidence quality that coffee protects against HCC as determined with GRADE was "very low" (table 3).

Table 3. GRADE Summary of Findings table.

An extra two cups of coffee per day for preventing HCC

Patient or population: risk of HCC
Setting: primary/secondary care
Intervention: two extra cups of coffee per day
Comparison: usual coffee consumption

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with no coffee	Risk with coffee				
HCC assessed with: cancer registries, death records and medical records	**High**		**RR 0.65** (0.59 to 0.72)	1.825 cases 2,905 controls 2115/1683071 exposed 654/399566 unexposed (26 observational studies)	⨁◯◯◯ VERY LOW [1]	The RR corresponds to two extra cups of coffee per day.
	50 per 1000	**33 per 1000** (30 to 36)				

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

1. The quality of evidence rating was downgraded because of (1) risk of bias (2) indirectness and (3) publication bias.

The effect of pre-existing CLD and HCC risk factors

Three cohort studies[26-28] performed sub-group analyses stratified by presence/absence of baseline CLD, which was poorly defined but included cirrhosis. Data from two of those studies showed an inverse association of coffee and HCC in those with baseline CLD but not without, whilst the other showed an inverse association without baseline CLD only. The pooled difference between the stratified estimates was not statistically significant (p=0.87). Data from a fourth (case-control) study[38] showed statistically significant inverse

associations between coffee and HCC, both when cases were compared to community controls and controls with CLD, 22% of whom had cirrhosis. Three other case-control studies[33, 35, 39] showed inverse associations between coffee and HCC using only controls with liver disease.

Results from the investigation into the influence of risk factors on the association between coffee and HCC are presented as supplementary information. In summary, there was no statistically significant difference in the associations between coffee and HCC according to viral hepatitis status, BMI, T2DM, or alcohol consumption.

Caffeinated and decaffeinated coffee

Four studies reported RRs of HCC specifically for decaffeinated coffee consumption.[12, 18, 32, 36] No single study reported a statistically significant association between HCC and decaffeinated coffee consumption. Three cohort studies,[12, 18, 32] involving approximately 750,000 participants and 800 cases, reported dose-response RRs or RRs for >2 consumption categories. The pooled RR of HCC for two extra cups per day was 0.86 (95% CI 0.74-1.00; three studies). Only two studies, involving approximately 850,000 participants and 900 cases, reported RRs of HCC according to caffeinated coffee consumption in a manner suitable for dose-response analysis.[12, 32] The pooled RR of HCC for an extra two cups of caffeinated coffee was 0.73 (95% CI 0.63-0.85).

DISCUSSION

In our meta-analysis of 18 cohort studies, involving 2,272,642 participants and 2,905 cases, and 8 case-control studies, involving 1,825 cases and 4,652 controls, increasing coffee consumption by two cups per day was associated with a 35% reduction in the risk of HCC (RR 0.65; 95% CI 0.59-0.72). This is similar to previous meta-analyses.[10, 20] In a subset of studies, the association was not significantly different in participants with pre-existing CLD

at baseline, some of whom had cirrhosis. This is an important finding as the absolute risk of HCC in cirrhosis is high but may be more than halved by 5 cups/day of coffee compared to none (figure 3). The association was also not significantly different for the main exposures for HCC: high alcohol consumption, high BMI, T2DM, and HBV/HCV.[40] Data from the few studies which specified coffee type showed that increasing caffeinated and decaffeinated coffee consumption by two cups per day were associated with reductions of 27% (RR 0.73, 95% CI 0.63-0.85) and 14% (RR 0.86, 95% CI 0.74-1.00) in the risk of HCC. This is the strongest evidence to date of an association between decaffeinated coffee and HCC. It has importance for developing coffee as a lifestyle intervention in CLD, as decaffeinated coffee might be more acceptable to those who do not drink coffee or who limit their coffee consumption because of caffeine related symptoms.

Other major strengths of this meta-analysis are the systematic approach used to calculate a dose-response between coffee and HCC and the inclusion of a large number of participants and cases, representing a range of demographic groups (e.g. gender, nationality etc.) and the main risk factors for HCC. We were able to show that there was no effect modification by baseline CLD and HCC aetiology.

The main limitation is that all the included studies were observational and, thus, we cannot infer causation. Observational studies are susceptible to bias and confounding, and case-control studies are at particular risk of selection and information bias. In the case-control studies, cases were mostly from hospital admissions or clinic records, which may not be representative of all HCC. Not all patients with HCC are admitted to hospital, and individual factors associated with likelihood to attend clinic and/or to participate in a research study may be associated with coffee consumption or other risk factors (and confounders) for HCC. In addition, because of the need to interview participants, dead cases were not included.

The use of hospital controls in all except one study may also have introduced bias. Firstly, there are associations between coffee drinking and a large number of other health conditions.[41] Second, hospitals vary in the scale of their catchment areas and so hospital controls may not be representative of the populations from which cases arose especially in areas where HCC care is highly specialised.

Among the cohorts, some studies used primary liver cancer as an outcome, whereas others used HCC. All but one cohort study used cancer registries to identify cases, sometimes in combination with death records. Cancer registries are more robust for ascertainment than death records.

Residual confounding likely existed in all studies from hidden factors and misclassification of measured confounders. However, adjustment for confounders had minimal effect on the association between coffee and HCC suggesting residual effects will be small. All studies adjusted for alcohol, but several did not adjust for BMI, T2DM and HBV/HCV. Coffee was associated with alcohol in some studies, so failure to capture alcohol robustly might underestimate the inverse association between coffee and HCC.[12, 30] The cohorts generally did not adjust for HBV/HCV despite it being a major risk factor for HCC, but prevalence was likely low and we found no evidence of an effect of HBV/HCV infection on the association between coffee and HCC.

The measurement of coffee consumption may also have introduced bias in case-control studies due to recall bias. Belief that coffee was harmful may have led to overestimation of consumption in cases. However, cases may have reduced coffee consumption because liver disease slows caffeine metabolism.[42] One study used for baseline the consumption at two years before HCC diagnosis[36] when decades before may have been more appropriate. Another study[35] reported RRs of HCC according to consumption pre- and post-

identification of liver disease, the weaker pre-identification estimates were used in the meta-analysis, with minimal effect on the overall pooled RR.

In the cohorts, baseline CLD may have been present in cases given the short follow-up time of some cohorts compared to the long time for HCC to develop. However, we looked at a number of cohorts that presented data stratified by baseline CLD status and found no significant effect on the association between coffee and HCC. Setiawan et al. found that the RR of HCC for two or more cups of coffee daily compared to none remained comparable in magnitude and statistically significant when deaths in the first two years were excluded. Lai et al. found that the RR of HCC for an extra cup of coffee per day was 0.81 (95% CI 0.66-0.98) in the first ten years and 0.83 (95% CI 0.71-0.96) in the final ten years of the study. Bamia et al.[18] Hu et al.[29] and Shimazu et al.[28] reported similar findings. Thus, drinking coffee appeared to protect against HCC in participants with varying levels of undiagnosed CLD at baseline.

Our method of estimating median consumption in the reported consumption categories may have exaggerated the effect size.

There was statistically significant heterogeneity between the studies; in a meta-regression analysis, it was not significantly associated with publication year, length of follow-up (cohorts only), percentage of alcohol abstainers, age or gender of participants.

Heterogeneity might be due to how consumption of coffee was measured. The included studies asked participants to estimate coffee consumption, usually by selecting from a list of predefined categories in food frequency questionnaires (FFQs). Different categories may have influenced participants' responses. There may be variation in the size of cups, preparation (e.g. boiled vs. filtered) and caffeine content; "coffee" was taken to be the pattern of use prevalent in the particular study population. Proportions of decaffeinated coffee

drinkers varied markedly and were very low in certain countries (e.g. Japan and Finland).[29, 38] Higher proportions of decaffeinated coffee drinkers, such as in the United States,[32] may have attenuated the overall effect size given the weaker association found here between decaffeinated coffee and HCC.

Language bias cannot be excluded since we only included English studies, although studies found in the search were mostly in English. Generally, evidence of a significant influence in meta-analyses of language bias is weak.[43] Studies published in non-English journals may also be less rigorous and report bigger effect estimates.[44] Thus, our inclusion of English studies only is not likely to have introduced significant bias. Finally, we found evidence of publication bias using Egger's test. Adjusting for smaller unpublished studies pushed the effect size towards null but it remained statistically significant.

Mechanism of action

As discussed in detail in our previous work,[15, 45] there is biological plausibility of a protective effect of coffee against HCC. The fact we found no significant effect of aetiology albeit in a subset of studies suggests that the apparent protective mechanism acts via a common pathway, such as the development of cirrhosis. Eighty to 90% of cases of HCC develop on a background of cirrhosis,[45] and several studies and a meta-analysis have reported an inverse association between coffee and cirrhosis.[15] Our findings suggest a central role for caffeine, given that the association was weaker for decaffeinated coffee. Non-coffee sources of caffeine are also inversely associated with HCC.[46] Caffeine is a non-selective antagonist of adenosine receptor A2aAR.[47] A2aAR activation substantially enhances synthesis of collagen by hepatic stellate cells (HSCs).[48] Caffeine also reduces activity of tumour necrosis factor-α, an inflammatory agent, and down-regulates reactive

oxygen species production by Kupffer cells.[49] Thus, caffeine might suppress the development of cirrhosis by reducing overall oxidative stress and inflammation.

However, our findings provide support for a protective effect of decaffeinated coffee against HCC, and other observational studies have reported that decaffeinated coffee is inversely associated with cirrhosis and abnormal LFTs.[12, 50] Other biologically active ingredients in coffee include chlorogenic acid, kahweol and cafestol, and there is some evidence that these may confer protection against liver fibrosis.[11]

Coffee may also possess anti-carcinogenic properties in addition to the prevention of cirrhosis. This is supported by our finding that the association of coffee and HCC was seen in those with pre-existing CLD, including cirrhosis. Caffeine reduces HCC cell proliferation.[51] Cafestol and kahweol increase activity of phase 2 liver enzymes, which may improve metabolism and excretion of carcinogens,[52, 53] and compounds including polyphenols may ameliorate oxidative DNA damage. However, cafestol and kahweol are present only in minimal quantities in instant and filtered coffee,[54] and these varieties are popular in Japan and Finland, respectively, where studies included in this meta-analysis show inverse associations with HCC.[29, 38]

Other specific mechanisms of protection might include inhibition of hepatitis virus activity[55] and prevention of T2DM.[29]

Coffee purportedly possesses a range of health effects in addition to those on the liver, including lower incidences of neurological diseases, various cancers and any-cause mortality.[41] However, randomised trials are needed of interventions to support patients at risk of HCC to increase coffee consumption before recommending an increase given the examples in other areas of where RCTs have shown observational data to be incorrect and the global scale and ubiquity of coffee consumption.[56] The potential harms of coffee also

require further investigation including the reported increased risk of lung cancer and bone fractures[41] and the deleterious effect on cholesterol, which could potentially exacerbate the already increased risk of CVD associated with certain types of liver disease.[57]

In summary, this study has shown that an extra two cups of coffee per day is associated with a one-third reduction in the RR of HCC. Our findings are significant given the increasing incidence of HCC, and the overall poor prognosis of this condition. Randomised trials should investigate the effectiveness of increasing coffee consumption in those at risk of HCC including patients with existing CLD.

COMPETING INTERESTS

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

ETHICS APPROVAL

Not required.

FUNDING

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DATA SHARING

No additional data are available.

CONTRIBUTORSHIP

The study was conceived by all authors; The search was performed by OJK; The studies were reviewed and selected by RB and OJK; The quality of evidence assessment was performed by OJK; The risk of bias assessment was performed by JP and OJK; The data was extracted and checked by OJK and RB, respectively; The statistical analysis was performed by OJK; The manuscript was drafted by OJK and reviewed and amended by all authors. JP is guarantor.

FIGURES

Figure 1. An illustration showing how the studies included in this meta-analysis were reviewed and selected.

Figure 2. A forest plot illustrating RRs of HCC for an extra two cups of coffee per day. The RRs as reported by the individual studies are shown as squares. The sizes of the squares represent the weightings in the random-effects model. The pooled RRs (from cohorts, case-control studies and all studies) are shown as diamonds.

Figure 3. Results of a cubic spine dose-response meta-analysis of the association between coffee and HCC.

Figure 4. Filled funnel plot for the risk of HCC per extra two cups of coffee daily. Black circles represent the included studies found by our search, while white circles represent the "missing" unpublished studies detected in the trim-and-fill analysis.

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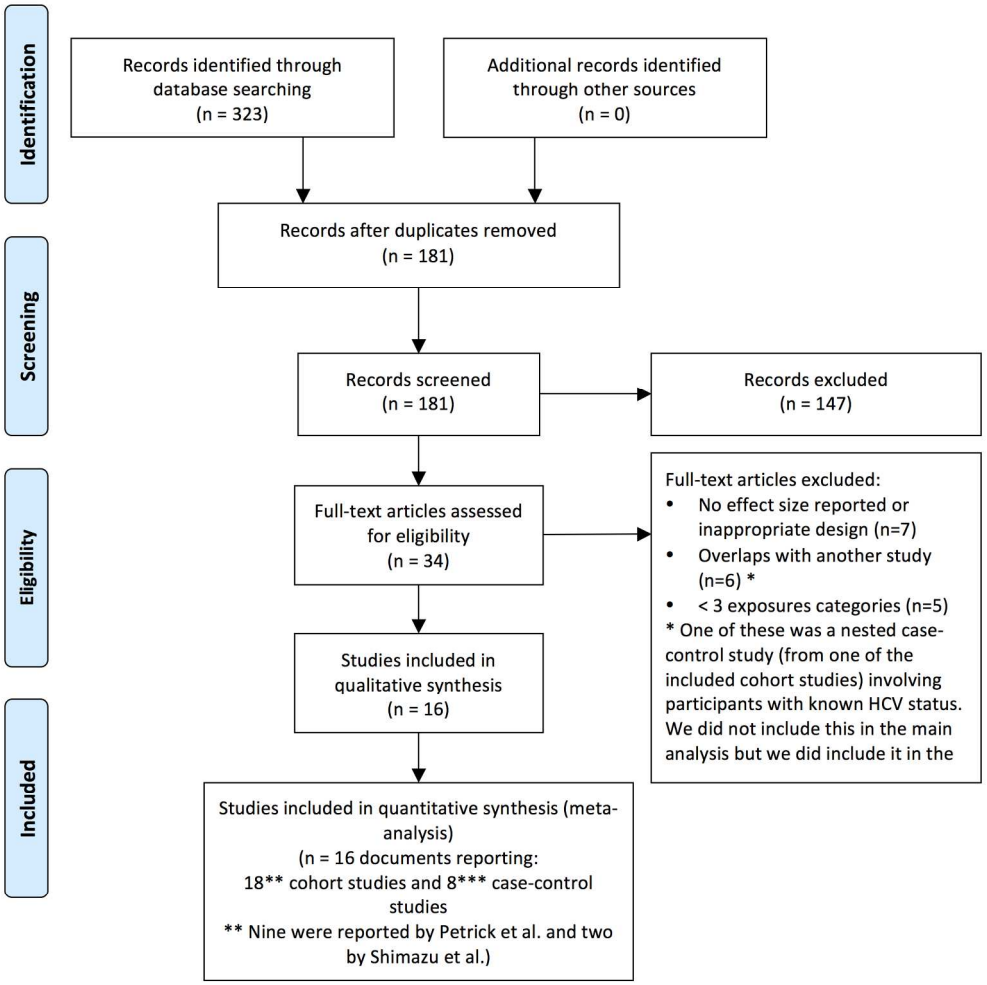

Figure 1. An illustration showing how the studies included in this meta-analysis were reviewed and selected.

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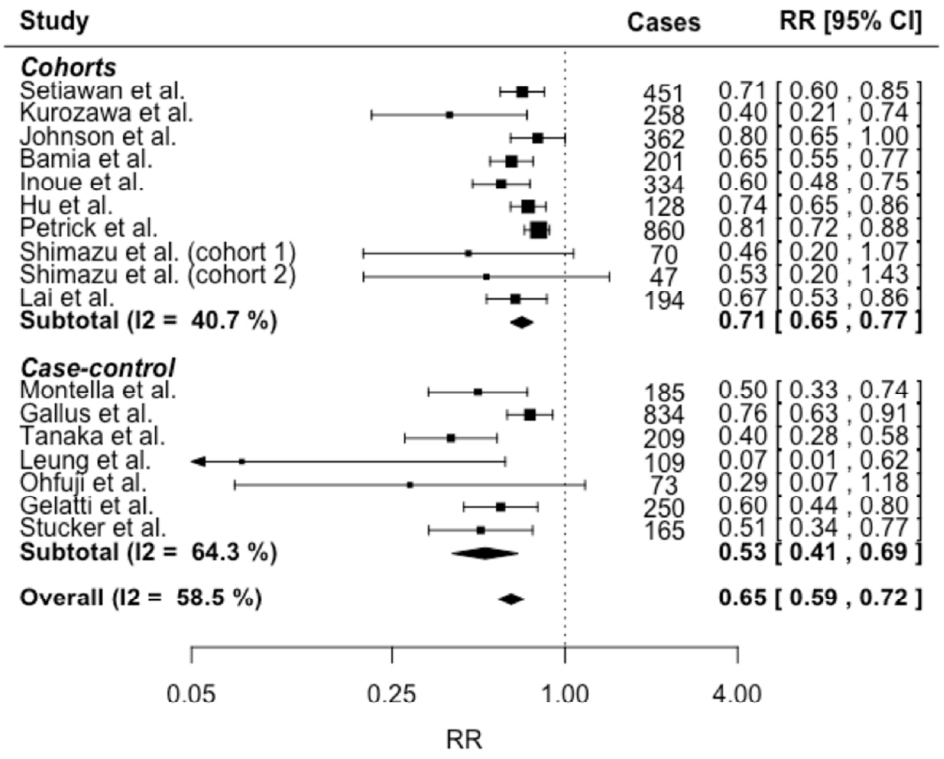

Figure 2. A forest plot illustrating RRs of HCC for an extra two cups of coffee per day. The RRs as reported by the individual studies are shown as squares. The sizes of the squares represent the weightings in the random-effects model. The pooled RRs (from cohorts, case-control studies and all studies) are shown as diamonds.

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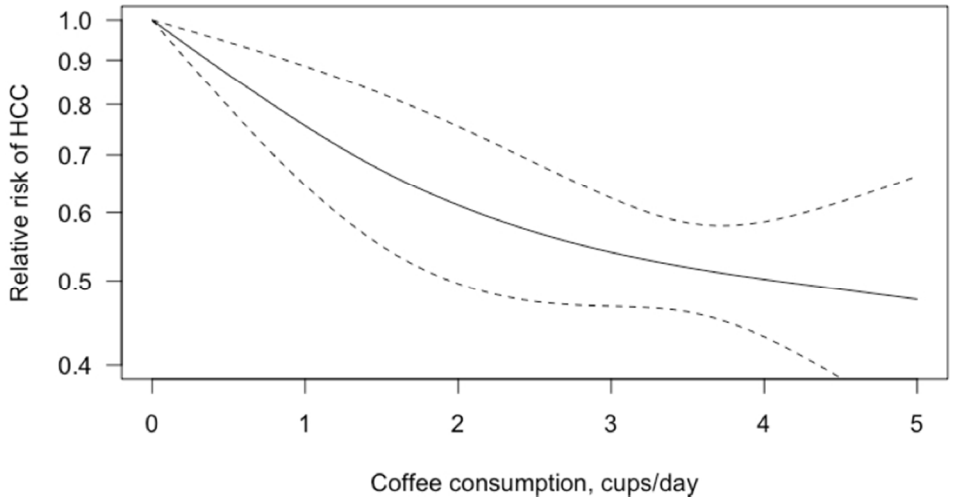

Figure 3. Results of a cubic spine dose-response meta-analysis of the association between coffee and HCC.

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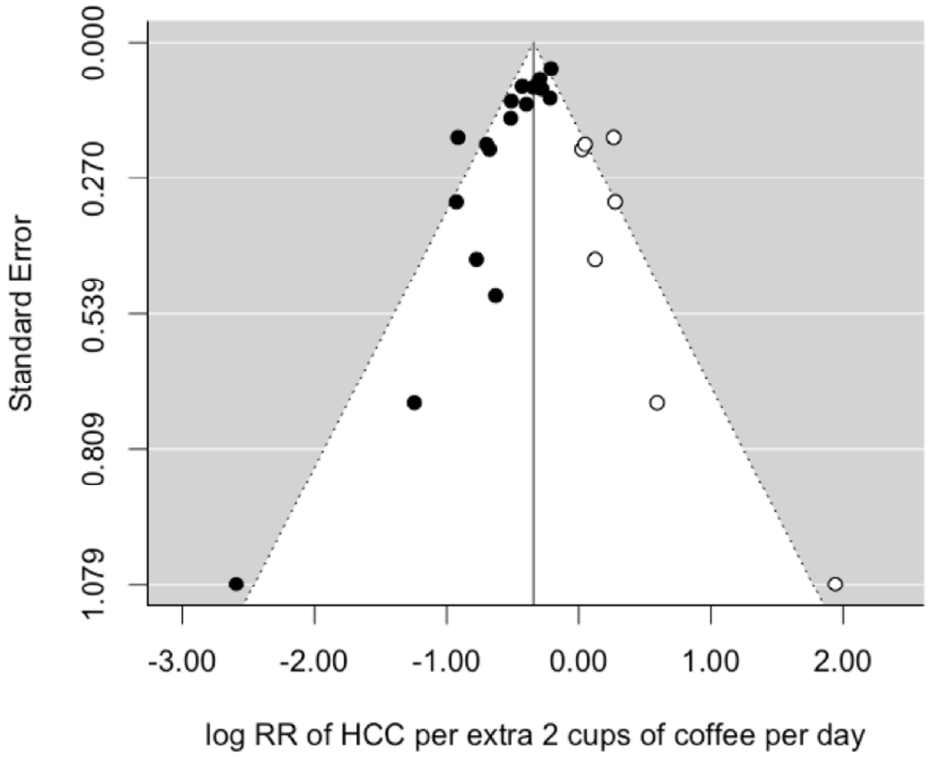

Figure 4. Filled funnel plot for the risk of HCC per extra two cups of coffee daily. Black circles represent the included studies found by our search, while white circles represent the "missing" unpublished studies detected in the trim-and-fill analysis.

159x146mm (150 x 150 DPI)

Supplementary Information: Caffeinated and Decaffeinated Coffee Consumption and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis

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THE ASSOCIATION BETWEEN COFFEE AND HCC ACCORDING TO RISK FACTOR EXPOSURE

Viral hepatitis B and C

Individual studies reported statistically significant reductions in the RR of HCC with increasing coffee consumption in participants who were HBV positive,[1] HCV positive[2] and negative for both.[3] Three studies reported RRs stratified by HBV/HCV status in a manner suitable for dose response analysis.[2-4] One of those studies reported RRs in a subgroup with HCV,[2] one in a subgroup with HBV and/or HCV[3] and one in two subgroups with (i) HBV or (ii) HCV[4] (some participants were co-infected and in both subgroups). The pooled RR of HCC for an extra two cups of coffee per day with HBV/HCV was 0.59 (95% CI 0.34-1.00; three studies) and 0.56 (95% CI 0.42-0.74; three studies) when we included the HBV and HCV

estimates, respectively, from the study with separate subgroups. Both those were weaker than the corresponding RR without HBV/HCV of 0.42 (95% CI 0.26-0.70; three studies) but in neither case was the difference statistically significant.

Diabetes and BMI

Two studies reported RRs of HCC according to coffee consumption stratified by diabetes status.[5, 6] For both studies, the RRs for an extra two cups of coffee per day were statistically significant for participants without but not with diabetes, although this may have been due to small sample size for DM. The pooled RR of HCC for an extra two cups of coffee per day was 0.79 (95% CI 0.72-0.86; two studies) without diabetes, which was larger than the corresponding RR of 0.84 (95% CI 0.69-1.04; two studies) with diabetes. The difference was not statistically significant.

Four studies reported RRs of HCC according to coffee consumption stratified by BMI.[5-8] The RRs for an extra two cups of coffee per day were statistically significant in two of the four studies in both the highest (above 25 and 30 kg/m2) and lowest (below 25 and 30 kg/m2) BMI categories.[6, 8] For the other studies, the RRs were statistically significant in the highest BMI category only (above 25 kg/m2 for both).[5, 7] In all four studies, the associations were stronger in the highest BMI category than the lowest. The pooled RR for an extra two cups of coffee per day was 0.72 (CI 95% 0.63-0.81; four studies) in the highest BMI category, which was larger than the corresponding RR in the lowest of 0.78 (95% CI 0.71-0.86; four studies). The difference was not statistically significant.

Alcohol consumption

Five studies reported RRs of HCC according to coffee consumption stratified by alcohol intake in a manner suitable for dose-response analysis.[3-5, 8, 9] The RRs of HCC for an extra two cups of coffee per day were statistically significant in three studies for the highest categories

of alcohol consumption[3, 4, 8] and in three studies for the lowest.[5, 8, 9] The pooled RR of HCC for an extra two cups of coffee per day in the highest category of alcohol consumption was 0.63 (95% CI 0.51-0.77; five studies) compared to 0.71 (95% CI 0.63-0.79; five studies) in the lowest. The difference was not statistically significant.

PRISMA-P PROTOCOL

Section and topic	Checklist item
ADMINISTRATIVE INFORMATION	
Identification	We will perform a systematic review with meta-analysis of the relationship between caffeinated and decaffeinated coffee and hepatocellular carcinoma (HCC). There are existing meta-analyses on coffee and HCC but none on decaffeinated coffee or the influence of HCC aetiology.
Registration	Our protocol is unregistered
Authors	Oliver John Kennedy[1]; Paul Roderick[1], Ryan Buchanan[1], Jonathan Fallowfield[2], Peter Hayes[2], Julie Parkes[1] 1. Primary Care & Population Sciences Faculty of Medicine University of Southampton 2. MRC Centre for Inflammation Research University of Edinburgh
Support	There are no sponsors or financial interests to declare.
INTRODUCTION	
Rationale	Primary liver cancer is the sixth most commonly diagnosed cancer worldwide. Hepatocellular carcinoma (HCC) is the most common subtype of primary liver cancer. Of concern is the global increase of non-alcoholic steatohepatitis/metabolic syndrome which can progress to HCC in the absence of cirrhosis. A number of studies have shown drinking coffee is inversely associated with the risk of diseases affecting the liver, including HCC.
Objectives	To determine quantitatively the relationship between caffeinated and decaffeinated coffee and the risk of HCC. To investigate whether the relationship between coffee and HCC is influenced by pre-existing liver disease or specific risk factors (e.g. EtOH, HBV/HCV, metabolic factors)
METHODS	
Eligibility criteria	We will include studies in our meta-analysis that: • are cohort or case-control studies • report effect sizes (RRs, OR, HRs) for primary liver cancer/HCC according to coffee intake (adults only). We will exclude studies that: • report no dose-response or provide insufficient information for one to be computed. • are published in a language other than English.

Information sources	Searches will be performed for published studies using Web of Science, Pubmed and Embase, and no limitation of date of publication will be imposed. Manual searches of reference lists will be performed.
Study selection process	Duplicates will be removed before two authors screen studies, first by abstracts and titles followed by full text.
Data collection process	The following data will be extracted from the included studies: • first author, date of publication, country, the design of the study, the exclusion and inclusion criteria, the estimates and adjustments, the numbers of participants (or controls) and cases, the methods of measuring exposure and case identification, cohort follow-up (time, losses), whether baseline liver disease was excluded. • the most rigorously adjusted effect sizes and effect sizes stratified by pre-existing chronic liver disease, alcohol consumption, BMI, hepatitis B and C virus status, diabetes, and type of coffee. • RRs for total caffeinated and decaffeinated coffee consumption, including RRs stratified by pre-existing liver disease and aetiology.
Data items	We will assume that hazard ratios, odds ratios and relative risks are the same.
Outcomes and prioritization	HCC stratified by risk factors / aetiology / type of coffee
Risk of bias	Newcastle-Ottawa scale shall be used for risk of bias assessment
Data synthesis	We will calculate RRs for a two cups/day increase and for 1-5 cups per day, where possible stratified by risk factors. I2 and Cochrane Q will be used to assess heterogeneity. We will re-run the analysis while omitting each study.
Additional analyses	Eggers test and a trim-and-fill analysis will be used to investigate publication bias.
Confidence in cumulative evidence	GRADE will be used for assessment of evidence quality

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Research Checklist: Caffeinated and Decaffeinated Coffee Consumption and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis

MOOSE CHECKLIST FOR META-ANALYSES OF OBSERVATIONAL STUDIES.

	Page/table/figure	Details
Reporting of background should include		
Problem definition	4	Primary liver cancer is the sixth most commonly diagnosed cancer. HCC is the most common subtype of primary liver cancer. HCC usually develops in people with cirrhosis but HCC without cirrhosis is becoming more common because of the increasing prevalence of non-alcoholic steatohepatitis/metabolic syndrome.
Hypothesis statement	4	Coffee has been associated with a reduced risk of hepatocellular carcinoma (HCC). It is unclear whether the inverse association also exists for decaffeinated coffee or whether it is influenced by HCC aetiology.
Description of study outcomes	5	HCC (all causes)
Type of exposure or intervention used	5	Caffeinated and decaffeinated coffee consumption
Type of study designs used	5	Observational studies
Study population	5	All populations.
Reporting of search strategy should include		
Qualifications of searchers (eg librarians and investigators)	5	The authors did the searches
Search strategy, including time period used in the synthesis and key words	5	Search term: (“odds” OR “risk” OR “hazard” OR "OR" OR "RR" OR "HR") AND “coffee” AND (“liver” OR “hepatocellular*) AND (“cancer“ OR “carcino*” OR neoplas*). We placed no restrictions on publication dates.
Effort to include all available studies, including contact with authors	5	All studies eligible were included.
Databases and registries searched	5	Web of Science, Embase, and PubMed
Search software used, name and version, including special features used (eg explosion)	5	Ovid was used for searching Embase.
Use of hand searching	5	References of pertinent studies were

(eg reference lists of obtained articles)		searched manually.
List of citations located and those excluded, including justification	8, Figure 1	Figure 1 illustrates the process for selecting the studies for inclusion in this meta-analysis. We will provide citations for the excluded study by request.
Method of addressing articles published in languages other than English	5	English studies only.
Method of handling abstracts and unpublished studies	5	Only published studies (abstracts were not excluded).
Description of any contact with authors	5,7	Published data only.
Reporting of methods should include		
Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	5,6	Detailed exclusion and inclusion criteria are specified.
Rationale for the selection and coding of data (eg sound clinical principles or convenience)	6	The data extracted from each study related to study type, categories of exposure, outcome, adjustment for confounding and population characteristics.
Documentation of how data were classified and coded (eg multiple raters, blinding and interrater reliability)	5,6	One author extracted the data which was then checked for accuracy by a second author.
Assessment of confounding (eg comparability of cases and controls in studies where appropriate)	6	The risk of bias was investigated by the Newcastle-Ottawa scale. The direction and magnitude of overall adjustment was calculated.
Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	6	The risk of bias was investigated by two authors separately and the conclusions discussed for agreement.
Assessment of heterogeneity	6,7	Heterogeneity was assessed using I^2 and Cochran's Q.
Description of statistical methods (eg complete description of fixed or random effects models, justification of whether	7,8	We used a random effects meta-analysis after first calculating a RR of HCC for an increase in consumption of 2 cups/day for each study.

the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated		
Provision of appropriate tables and graphics	Figure 1	Figure 1 shows the search and study selection process.
Reporting of results should include		
Graphic summarizing individual study estimates and overall estimate	Figure 2	
Table giving descriptive information for each study included	9,13, Tables 1 and 2	
Results of sensitivity testing (eg subgroup analysis)	14,15	We report results of several sensitivity analyses investigating the effect of study type and study quality.
Indication of statistical uncertainty of findings	13	95% confidence intervals are provided / p-values are provided where necessary.
Reporting of discussion should include		
Quantitative assessment of bias (eg publication bias)	13	We used Egger's test and a trim-and-fill analysis.
Justification for exclusion (eg exclusion of non-English language citations)	8, Figure 1	Studies were excluded if they did not provide a dose-response estimate or allow one to be calculated.
Assessment of quality of included studies	8, Table 1	We test if the effect size varies when we restrict the analysis to high quality studies.
Reporting of conclusions should include		
Consideration of alternative explanations for observed results	16-19	We discuss residual confounding and possible sources of bias.
Generalization of the conclusions (eg appropriate for the data presented and within the domain of the literature review)	16	The vast majority of the cohort studies were of the general population.
Guidelines for future research	21	Last paragraph in the main text.
Disclosure of funding source	21	See " FUNDING" section.

BMJ Open

Coffee, Including Caffeinated and Decaffeinated Coffee, and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis

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Keywords:	Coffee, Hepatocellular carcinoma, Liver disease, Meta-analysis

Coffee, Including Caffeinated and Decaffeinated Coffee, and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis

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KEYWORDS: coffee, hepatocellular carcinoma, HCC, liver disease, meta-analysis

WORD COUNT: 4102 (main text)

ABSTRACT

Objectives: To examine the association between coffee, including caffeinated and decaffeinated coffee, with hepatocellular carcinoma (HCC) and assess the influence of HCC aetiology and pre-existing liver disease.

Design: We performed a systematic review and meta-analysis. We calculated relative risks (RRs) of HCC according to caffeinated and decaffeinated coffee consumption using a random-effects dose-response meta-analysis. We tested for modification of the effect estimate by HCC aetiology and pre-existing liver disease. We judged the quality of evidence using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) criteria.

Results: We found 18 cohorts, involving 2,272,642 participants and 2,905 cases, and 8 case-control studies, involving 1,825 cases and 4,652 controls. An extra two cups/day of coffee was associated with a 35% reduction in the risk of HCC (RR 0.65, 95% CI 0.59-0.72). The inverse association was weaker for cohorts (RR 0.71, 95% CI 0.65-0.77), which were generally of higher quality than case-control studies (RR 0.53, 95% CI 0.41-0.69). There was evidence that the association was not significantly altered by stage of liver disease or the presence/absence of high alcohol consumption, high body mass index, type-2 diabetes mellitus, smoking or hepatitis B and C viruses. An extra two cups of caffeinated and decaffeinated coffee (2 and 3 cohort studies, respectively) were associated with reductions of 27% (RR 0.73, 95% CI 0.63-0.85) and 14% (RR 0.86, 95% CI 0.74-1.00) in the risk of HCC. However, due to a lack of randomised controlled trials, potential publication bias and there being no accepted definition of coffee, the quality of evidence under the GRADE criteria was "very low".

Conclusions: Increased consumption of caffeinated coffee and, to a lesser extent, decaffeinated coffee is associated with reduced risk of HCC, including in pre-existing liver disease. These findings are important given the increasing incidence of HCC globally and its poor prognosis.

ARTICLE SUMMARY

Strengths and limitations of this study

Strengths:

- This is the first meta-analysis to calculate RRs of HCC for 1-5 cups of coffee per day, which may be useful in the design of a coffee based intervention for evaluation in a clinical trial.
- This is the first meta-analysis to investigate the influence of all the main HCC risk factors on the association between coffee and HCC.
- This is the first meta-analysis to calculate a RR of HCC for decaffeinated coffee consumption.

Limitations:

- There was heterogeneity between the studies included in the meta-analysis.
- Many studies did not specify coffee caffeine content.

INTRODUCTION

Primary liver cancer is the sixth most commonly diagnosed cancer worldwide and, because of its poor prognosis, the second leading cause of cancer death.[1, 2] Hepatocellular carcinoma (HCC) is the dominant histological subtype accounting for 85-90% of cases.[3] HCC most commonly develops in people with cirrhosis due to chronic viral hepatitis B (HBV) or C (HCV), excess alcohol consumption and/or non-alcoholic fatty liver disease (NAFLD).[3] Non-alcoholic steatohepatitis (NASH), which is rapidly increasing worldwide, can lead to the development of HCC in the absence of cirrhosis.[4] The incidence of liver cancer is increasing due to changes in these underlying risks and by 2030 the number of new cases annually will have risen by around 50% to over 1.2 million.[5] The burden of liver cancer is highest in East and South East Asia, with China alone accounting for 50% of cases worldwide.[2] Only 10%-37% of patients diagnosed with HCC are eligible for potentially curative tumour resection (partial hepatectomy).[6] Thus, prognosis remains poor with a 5-year overall survival rate of 18%.[7]

Coffee is a popular drink in most countries with approximately 2.25 billion cups consumed daily.[8] It is a complex mixture of biologically active molecules, including caffeine, chlorogenic acid and diterpenes.[9] These compounds possess anti-oxidant, anti-inflammatory anti-fibrotic and anti-carcinogenic properties which may explain observational data that coffee drinkers have lower rates of chronic liver disease (CLD), including fibrosis, cirrhosis and HCC.[10] Reports by the World Cancer Research Fund[11] and the International Agency for Research on Cancer[12] are both supportive of a protective role of coffee against HCC. In addition, a recent meta-analysis reported that the relative risk (RR) of HCC for an extra cup of coffee per day was 0.74 (95% CI 0.65-0.83).[13] However, to date no randomised controlled trials investigating a coffee intervention for preventing HCC have been performed. Challenges in designing such a trial include a lack of understanding of the

effect modification by aetiology or risk factors for HCC (e.g. alcohol liver disease, NASH, cirrhosis etc.). In addition, there is uncertainty as to whether all types of coffee are equally beneficial, especially given their differing chemical compositions (e.g. caffeinated vs. decaffeinated coffee). To help address these challenges, we have now explored, for the first time in a meta-analysis, the modification of the inverse association between coffee and HCC by key risk factors, such as HBV/HCV, high body mass index (BMI), type-2 diabetes mellitus (T2DM), smoking, alcohol consumption and the presence of CLD including cirrhosis. We also report the first meta-analysis for the association between decaffeinated coffee and HCC. Decaffeinated coffee protects against liver damage in animal studies[14] and is inversely associated with T2DM, abnormal liver function tests (LFTs) and cirrhosis in human observational studies.[15-17]

METHODS

The methods used were similar to those described in our earlier work,[18] and are detailed below. We followed the PRISMA guidelines; a protocol, which was pre-specified but not pre-registered online, is provided as supplementary information.

Searches and selection of studies

We performed searches of abstracts and titles in Web of Science, Embase, and PubMed with: (“odds” OR “risk” OR “hazard” OR "OR" OR "RR" OR "HR") AND “coffee” AND (“liver” OR “hepatocellular*”) AND (“cancer“ OR “carcino*” OR “neoplas*”). The searches were run in September 2015 without restriction of date of publication. References of pertinent studies were searched manually. After removing duplicates, OJK and RB independently screened the titles and abstracts of the studies found in the search. Studies were included that: (i) reported a RCT, case-control study or cohort study; and (ii) reported hazard ratios (HRs), odds ratios (ORs) or relative risks (RRs) with 95% confidence intervals (95% CIs) for HCC

in adults according to consumption of coffee. Studies were excluded that i) did not report a dose-response or give sufficient information for calculation of a dose-response (i.e. this requires estimates for more than two exposure levels, or ii) were in a non-English language. We assumed cases of primary liver cancer to be HCC. If studies overlapped, we included the largest study or otherwise the last published study. We worked from published studies only, including abstracts, although we unsuccessfully attempted to acquire unpublished data from the authors of one study, as indicated below.

Extraction of data and assessment of quality

We extracted the following information from each study: the first author, the date of publication, the geographic region, the design of the study, the exclusion and inclusion criteria, the estimates and adjustments, the numbers of participants (or controls) and cases, the methods of measuring exposure and case identification. We also extracted data concerning cohort follow-up (time, losses) and whether baseline liver disease was excluded. We extracted the most rigorously adjusted effect sizes. We extracted effect sizes stratified by pre-existing CLD, smoking status, alcohol consumption, BMI, hepatitis B and C virus status, T2DM, and type of coffee. OJK extracted the data which RB then checked. Given the low incidence of HCC, we considered ORs, RRs, HRs to be equivalent, and for simplicity we use RR to refer to all three herein. We assessed the quality of the included studies using the Newcastle-Ottawa Scale.[19] We judged the quality of evidence with Grading of Recommendations Assessment, Development and Evaluation (GRADE).[20]

Statistical methods

Coffee and HCC

Most studies did not distinguish caffeinated vs. decaffeinated coffee, so coffee was taken to be the pattern of use prevalent in the particular study population. We considered consumption

in cups, where necessary[21] converting millilitres into cups of 150 mL. For each study, we calculated a RR for an extra two cups per day using dose response data where available [22, 23] or by estimating the dose-response using the method of Greenland and Longnecker[24]. The unit of an "extra two cups" per day was selected to represent a potential coffee based intervention, which could be used in clinical trials, and to maintain comparability with a previous meta-analysis [25]. We estimated median consumption for each reported consumption category to be the midpoint of closed ranges and the midpoint added to the amplitude of the previous range for open ranges.[25] We assessed whether the dose-response was non-linear by a cubic spline meta-analysis.[26] We tested for statistical heterogeneity using I^2 and Cochran's Q,[27] and interpreted p-values of <0.1 as statistically significant (for heterogeneity only) and we interpreted the I^2 values according to the chapter 9.5.2 of the Cochrane handbook.[27] We investigated heterogeneity by meta-regression and examined the impact of individual studies by re-running the analysis while leaving the studies out one at a time.[28] We tested for publication bias using Egger's test and a "trim-and-fill" analysis,[29] which we used to adjust the estimate for missing studies if publication bias was indicated. To assess the magnitude and direction of adjustment, we calculated a pooled unadjusted effect sizes for comparison with the corresponding adjusted effect size. We used random effects models (DerSimonian-Laird) and a two sided p-value of >0.05 for statistical significance. We used R (R Foundation for Statistical Computing, Vienna, Austria) with the metafor [30] and dosresmeta [31] packages for the analyses.

Effect modification by risk factors

We calculated RRs of HCC according to coffee consumption in participants stratified by baseline CLD. We also calculated and meta-analysed RRs stratified by exposure to each of: viral hepatitis status (carriers of HBV/HCV vs. negative for both), BMI (highest vs. lowest BMI categories), T2DM (presence vs. absence), alcohol consumption (highest vs. lowest

categories) and smoking (current smoker vs. ex/non-smoker). For these analyses, we only included studies that provided RRs for both exposed and non-exposed to the risk factors. Where available,[22, 23] we used dose-response data to calculate RRs for an increase in two cups of coffee per day. Otherwise, we used the Greenland and Longnecker method[24] where the number of exposed and non-exposed were provided[32-35] and variance-weighted least squares regression where they were not[36-39]. For each risk factor, we calculated a p-value for its modifying effect on the association between coffee and HCC by meta-analysing the differences between the exposed and unexposed RRs from each study. We also calculated the τ^2 for each of these analyses.

Caffeinated and decaffeinated coffee and HCC

Where possible we extracted data separately for caffeinated and decaffeinated coffee and calculated pooled RRs of HCC per two extra cups/day of each. One study, Bamia et al.,[21] reported RRs of HCC according to decaffeinated coffee consumption for three qualitative categories: “non-consumers”, “consumers below the median” and “consumers at/above the median”. We were unable to get the corresponding quantitative values after contacting the authors so used those reported by another publication investigating the effect of decaffeinated coffee on oesophageal cancer in the same cohort.[40] As above, we used dose response data where available [22]. Otherwise, we calculated the dose-response using the Greenland and Longnecker method [24] where the numbers of exposed and non-exposed[15] were available and variance-weighted least squares regression where they were not [21].

RESULTS

Coffee consumption and HCC

Figure 1 shows the searches and the stages of the selection of studies. Once duplicates were removed, we screened the abstracts and titles of 181 studies. Of those, we reviewed 34

studies in their entirety. Table 1 summarises the characteristics of the 16 studies which we included in the main meta-analysis.[15, 21-23, 32, 33, 35-39, 41-45] The studies were published between 2002 and 2015. Seven were from Europe, five from Japan, two from the US and one from each of Hong Kong and Singapore. The cohort studies primarily involved general populations (e.g. randomly selected from population registries) except for Lai et al.,[23] which included male smokers only. Total follow-ups ranged from seven[39] to 24 years,[23] and linkage to cancer registries was generally used to identify cases and exclude baseline HCC. The case-control studies were hospital based, with only one[33] using community controls. Fifteen studies reported estimates according to "coffee" consumption, while two and four studies, respectively, reported estimates specifically for caffeinated and decaffeinated coffee. The quality scores ranged from 4 to 8 (table 1) and were generally higher for cohorts (mean=6.9) compared to case-control studies (mean=5.0). A number of studies reported data from multiple cohorts or case-control studies. We extracted pooled estimates from Petrick et al.[22] (nine cohorts) and Gallus et al.[37] (two case-control studies) as equivalent study-specific estimates (e.g. in terms of adjustments for confounders and categories of coffee consumption) were not available. We extracted separate RRs from Shimazu et al[39] (two cohorts). Thus, this meta-analysis included data from 18 cohorts, involving 2,272,642 participants and 2,905 cases, and 8 case-control studies, involving 1,825 cases and 4,652 controls.

Table 1a. Details of the cohort studies meeting the inclusion criteria

Cohort study	Country	Population characteristics (age)	Cohort (% men)	Baseline exposure ascertainment	Outcome	Outcome ascertainment	Follow-up years	Cases (rate/1000)	NOS quality score
Inoue et al. 2005[36]	Japan	Gen pop (40-69)*	90,452 (48)	FFQ	HCC	Cancer registry, death records, medical records	9.7 (average)	334 (3.7)	7
Kurozawa et al. 2005[35]	Japan	Gen pop (40 to 79)*. HCC deaths within 1st 2 years excluded	110,688 (42)	FFQ	HCC death	Death records	9-11 (total)	258 (2.3)	7
Shimazu et al. (cohort 1) 2005[39]	Japan	Gen pop (≥40)*	22,404 (47)	FFQ	PLC	Cancer registry, death records, medical records	9 (total)	70 (3.1)	6
Shimazu et al. (cohort 2) 2005[39]	Japan	Gen pop (40-64)*	38,703 (49)	FFQ	PLC	Cancer registry, death records, medical records	7 (total)	47 (1.2)	6
Hu et al. 2008[38]	Finland	Gen pop (25-74)*	60,323 (49)	FFQ	PLC	Cancer registry	19.3 (median)	128 (2.1)	8
Johnson et al. 2011[41]	Singapore	Gen pop (45 to 74)*	61,321 (44)	FFQ	HCC	Cancer registry and death records	n/a	362 (5.9)	8
Lai et al. 2013[23]	Finland	Male smokers (50-69) from an RCT into lung cancer*. Self-reported cirrhosis excluded at baseline	27,037 (100)	FFQ	LC	Cancer registry	18.2 (median)	194 (7.2)	6
Bamia et al. 2014[21]	Europe **	Gen pop (25 to 70)*	486,799 (30)	FFQ	HCC	Cancer registry, death records, health insurance records and mail/telephone	11 (median)	201 (0.4)	7
Setiawan et al. 2015[15]	USA	Gen pop (45 to 75)*	162,022 (47)	FFQ	HCC	Cancer registry	18 (median)	451 (2.8)	7
Petrick et al. 2015[22]	USA	Gen pop (<50-≥70)*	1,212,893 (41)	FFQ	HCC	Cancer registry, medical records, self-reporting	Variable	860 (0.7)	6

Abbreviations: hepatocellular carcinoma (HCC), international classification of diseases (ICD), primary liver cancer (PLC), liver cancer (LC), intrahepatic cholangiocarcinoma (ICC), hepatitis B virus (HBV), hepatitis C virus (HCV), Newcastle-Ottawa Scale (NOS). * Participants with a diagnosis of HCC were excluded at baseline; ** Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom.

Table 1b. Details of the case-control studies meeting the inclusion criteria

Case-control study	Country	Case selection	N (% men) and age of cases	Control selection	N (%men) and age of controls	Measurement of coffee consumption	Outcome	NOS quality

								score
Gallus et al. 2002 (study 1)[37]	Italy	Hospital	501 (75) aged 20-75 (median 60)	Patients with non-cancer disorders in same hospital and from same catchment area	1552 (74) aged 18-75 (median 56)	FFQ	HCC	5
Gallus et al. 2002 (study 2)[37]	Greece	Hospital	333 (85) aged 31–79 (median 65)	Patients with non-cancer disorders in same hospital	360 (83) aged 24–79 (median 65)	FFQ	HCC	5
Gelatti et al. 2005[42]	Italy	Hospital	250 (82) aged less than 80 (mean 63.8)	Patients without liver disease in same hospital	500 aged less than 80 (mean 64.1)	FFQ	HCC	7
Ohfuji et al. 2006[43]	Japan	Attending hospital for HCV follow-up	73 (47) mean age 68.9	Attending hospital for HCV follow-up	253 (52) mean age 68.3	FFQ	HCC	5
Tanaka et al. 2007[33]	Japan	Hospital	209 (68) aged 40-79 (mean 67)	Community controls randomly selected	1308 (50) (mean 57)	FFQ	HCC	4
Montella et al. 2007[32]	Italy	Hospital	185 (81) aged 43-84 (median 66)	Patients in same hospital	412 (68) aged 40-82 (median 65)	FFQ	HCC	5
Leung et al. 2011[45]	Hong Kong	Attending hospital for HBV follow-up	109 (79) aged ≤39 to ≥60	Attending hospital for HBV follow-up	125 (82) aged ≤39 to ≥60	FFQ	HCC	5
Stucker et al. 2006[44]	France	Hospital	165 (100) aged <75	Patients without liver disease in same hospital	142 (100) aged <75	FFQ	HCC	4

The RRs of HCC according to coffee consumption are summarised in table 2, including adjustments for confounders. Most studies adjusted for age, alcohol and smoking, and a smaller number for HBV/HCV, BMI and T2DM. All the studies showed an inverse association between HCC for an extra two cups of coffee per day, although in four studies the relationship was not statistically significant. The pooled RR of HCC for an extra 2 cups/day across all studies for coffee was 0.65 (95% CI 0.59-0.72) (figure 2), for cohort studies it was 0.71 (95% CI 0.65-0.77) and for case-control studies 0.53 (95% CI 0.41-0.69). The pooled RR from studies with a quality score of 6 or above was 0.70 (95% CI 0.64-0.76) compared to 0.50 (95% CI 0.35-0.70) for those scoring below 6. The p-value for non-linearity of the dose-response was not statistically significant, and the pooled RRs for different levels of consumption of up to 5 cups per day are illustrated in figure 3. Adjustment for confounders had minimal effect, changing the pooled RR from 0.62 (95% CI 0.53-0.72) (i.e. unadjusted) to 0.65 (95% CI 0.59-0.72).

Table 2. The associations reported by the studies meeting the inclusion criteria for the main coffee-HCC meta-analysis.

Study	Coffee (cups per day, unless specified)	Participants	Cases (cumulative rate/1000)	Adjusted RR (95% CI)	Adjustments
Cohort studies					
Inoue et al. 2005[36]	Almost never 1-2/wk 3-4/wk 1-2 3-4 ≥5	29,423 17,159 10,316 23,753 7,316 2,485	161 (5.5) 65 (3.8) 36 (3.5) 54 (2.3) 15 (2.1) 3 (1.2)	1 (ref.) * 0.75 (0.56-1.01) * 0.79 (0.55-1.14) * 0.52 (0.38-0.73) * 0.48 (0.28-0.83) * 0.24 (0.08-0.77) *	Age, gender, alcohol, smoking, green tea, study area, green vegetable intake.
Kurozawa et al. 2005[35]	Non-drinkers <1 ≥1	24,556 15,259 44,151	103 (4.2) 57 (3.7) 98 (2.2)	1 (ref.) * 0.83 (0.54-1.25) * 0.5 (0.31-0.79) *	Age, gender, alcohol, smoking, T2DM, liver disease, education.
Shimazu et al. (cohort 1) 2005[39]	Never Occasionally ≥1	4,938 9,507 7,959	29 (5.9) 25 (2.6) 16 (2.0)	1 (ref.) ** 0.56 (0.33-0.97) ** 0.53 (0.28-1.00) **	Age, gender, alcohol, smoking, liver disease.
Shimazu et al. (cohort 2) 2005[39]	Never Occasionally ≥1	6,954 14,130 17,619	12 (1.7) 21 (1.5) 14 (0.8)	1 (ref.) ** 1.05 (0.52-2.16) ** 0.68 (0.31-1.51) **	Age, gender, alcohol, smoking, liver disease.
Hu et al. 2008[38]	0 to 1 2 to 3 4 to 5 6 to 7 ≥8	6,150 12,681 17,991 13,726 9,775	20 (3.3) 30 (2.4) 33 (1.8) 28 (2.0) 17 (1.7)	1 (ref.) * 0.66 (0.37-1.16) * 0.44 (0.25-0.77) * 0.38 (0.21-0.69) * 0.32 (0.16-0.62) *	Age, gender, alcohol, smoking, T2DM, liver disease, education, BMI, study year.
Johnson et al. 2011[41]	Non-drinkers 0-<1 1-<2 2-<3 ≥3	119,973 (PY) 70,762 (PY) 236,215 (PY) 190,567 (PY) 37,505 (PY)	69 38 149 92 14	1 (ref.) * 0.94 (0.63-1.40) * 1.17 (0.87-1.56) * 0.78 (0.56-1.07) * 0.56 (0.31-1.00) *	Age, gender, alcohol, smoking, T2DM, education, BMI, dialect group, year of recruitment, black and green tea.
Lai et al. 2013[23]	Never drinkers >0 to <1 1 to <2 2 to <3 3 to <4 ≥4 per extra cup	667 3,094 7,204 8,086 4,515 3,471	9 (13.5) 36 (11.6) 60 (8.3) 47 (5.8) 22 (4.9) 20 (5.8)	1.35 (0.65-2.82) ** 1 (ref.) ** 0.73 (0.48-1.12) ** 0.52 (0.33-0.82) ** 0.45 (0.26-0.78) ** 0.53 (0.30-0.95) ** 0.82 (0.73-0.93) **	Age, alcohol, smoking, T2DM, education, BMI, tea, cholesterol, marital status, ATBC intervention arm ¶.

Bamia et al. 2014[21]	Quintile 1 Quintile 2 Quintile 3 Quintile 4 Quintile 5	98,148 100,953 95,231 96,413 96,054	47 (0.5) 49 (0.5) 38 (0.4) 36 (0.4) 31 (0.3)	1 (ref.) * 0.85 (0.56-1.29) * 0.63 (0.39-1.02) * 0.49 (0.29-0.82) * 0.28 (0.16-0.5) *	Stratified for age and centre. Adjusted for gender, alcohol, smoking, T2DM, education, BMI, physical activity, energy intake, tea.
Setiawan et al. 2015[15]	Never <1 1 2 to 3 ≥4	44,438 31,056 45,717 32,593 8,218	119 (2.7) 111 (3.6) 137 (3.0) 67 (2.1) 17 (2.1)	1 (ref.) * 1.14 (0.88-1.48) * 0.87 (0.67-1.11) * 0.62 (0.46-0.84) * 0.59 (0.35-0.99) *	Age, gender, alcohol, smoking, T2DM, education, BMI, race.
Petrick et al. 2015[22]	Non-drinker >0 to <1 1 to <2 2 to 3 >3 per extra cup	172,950 164,977 179,781 370,786 161,116	85 (0.5) 138 (0.8) 149 (0.8) 255 (0.7) 97 (0.6)	1 (ref.) * 1.24 (0.94-1.64) * 1.16 (0.88-1.52) * 0.89 (0.68-1.15) * 0.73 (0.53-0.99) * 0.90 (0.85-0.94) *	Age, gender, alcohol, smoking, BMI, race, cohort.
Case-control studies		**Cases**	**Controls**		
Gallus et al. 2002 (Italian and Greek studies combined)[37]	Non drinkers 1 2 ≥3	129 231 292 178	256 432 582 637	1 (ref.) *** 1.2 (0.9-1.6) *** 1.0 (0.7-1.3) *** 0.7 (0.5-1.0) ***	Age, gender, alcohol, smoking, education, BMI, T2DM, hepatitis, study.
Gelatti et al. 2005[42]	No consumption 1 to 2 3 to 4 ≥5	44 119 69 18	59 206 163 72	1 (ref.) *** 0.8 (0.4-1.3) *** 0.4 (0.2-0.8) *** 0.3 (0.1-0.7) ***	Age, gender, alcohol, HBV, HCV.
Ohfuji et al. 2006[43]	Non drinkers <1 ≥1	25 19 29	63 74 116	1 (ref.) *** 0.61 (0.18-2.03) *** 0.38 (0.13-1.12) ***	Alcohol, smoking, BMI, duration of liver disease, disease severity, family history, interferon therapy, other caffeine containing beverage
Tanaka et al. 2007[33]	None Occasional 1 to 2 ≥3	127 53 17 12	268 496 268 221	1 (ref.) *** 0.33 (0.22-0.48) *** 0.27 (0.15-0.48) *** 0.22 (0.11-0.43) ***	Age, gender, alcohol, smoking, HBV, HCV.
Montella et al. 2007[32]	Abstainers <14/wk 14 to 20 21 to 27 ≥28	27 67 50 27 14	41 116 104 88 63	2.28 (0.99-5.24) *** 1 (ref.) *** 0.54 (0.27-1.07) *** 0.57 (0.25-1.32) *** 0.43 (0.16-1.13) ***	Age, gender, alcohol, smoking, education, centre, HBV, HCV.
Leung et al. 2011[45]	No coffee habit 1-3/wk ≥4 wk	86 11 12	82 17 26	1 (ref.) *** 0.58 (0.24-1.36) *** 0.41 (0.19-0.89) ***	Age, gender, alcohol, smoking, tea, physical activity
Stucker et al. 2006[44]	0-1 2	92 45	57 37	1 (ref.) *** 0.67 (0.3-1.3) ***	Alcohol

	>2	28	48	0.36 (0.2-0.7) ***	

Abbreviations: person years (PY), hepatitis B virus (HBV), hepatitis C virus (HCV), α-tocopherol or β-carotene (ATBC); * reported as HR; ** Reported as RR; *** Reported as OR. ¶ Participants were from another trial investigating vitamin E supplementation in the form of α-tocopherol or β-carotene;

Heterogeneity and sensitivity analysis

I^2 and the p-value for Cochran's Q were 58.5% and <0.01 respectively (figure 2), which indicated "moderate" to "substantial" between-study heterogeneity. Heterogeneity was lower for cohorts (I^2=40.7%; p=0.09) than case-control studies (I^2= 64.3%; p<0.01). In the sensitivity analysis, the RR was strongest when we excluded Hu et al.[38] (RR 0.63, 95% CI 0.56-0.71) and weakest when we excluded Tanaka et al.[33] (RR 0.68, 95% CI 0.62-0.74). Heterogeneity remained statistically significant throughout. In the meta-regression analysis, we found no statistically significant association of RR and publication year, length of follow-up (cohorts only), percentage of alcohol abstainers, age or gender.

Publication bias and quality of evidence

We found evidence of publication bias by Eggers test (p<0.0001) and visual inspection of the funnel plot as shown in figure 4. In our trim-and-fill analysis, we detected a number of "missing" smaller studies. Calibration for missing studies pushed the effect size of coffee towards null from 0.65 (95% CI 0.59-0.72) to 0.71 (95% CI 0.64-0.79). The evidence quality that coffee protects against HCC as determined with GRADE was "very low" (table 3).

Table 3. GRADE Summary of Findings table.

An extra two cups of coffee per day for preventing HCC

Patient or population: risk of HCC
Setting: primary/secondary care
Intervention: two extra cups of coffee per day
Comparison: usual coffee consumption

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with no coffee	Risk with coffee				
HCC assessed with: cancer registries, death records and medical records	**High**		**RR 0.65** (0.59 to 0.72)	1.825 cases 2,905 controls 2115/1683071 exposed 654/399566 unexposed (26 observational studies)	⨁◯◯◯ VERY LOW [1]	The RR corresponds to two extra cups of coffee per day.
	50 per 1000	**33 per 1000** (30 to 36)				

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

1. The quality of evidence rating was downgraded because of (1) risk of bias (2) indirectness and (3) publication bias.

The effect of pre-existing CLD and HCC risk factors

Three cohort studies[35, 36, 39] performed sub-group analyses stratified by presence/absence of baseline CLD, which was poorly defined but included cirrhosis. Data from two of those studies showed an inverse association of coffee and HCC in those with baseline CLD but not without, whilst the other showed an inverse association without baseline CLD only. The pooled difference between the stratified estimates was not statistically significant (p=0.87). Data from a fourth (case-control) study[33] showed statistically significant inverse

associations between coffee and HCC, both when cases were compared to community controls and controls with CLD, 22% of whom had cirrhosis. Three other case-control studies[37, 43, 45] showed inverse associations between coffee and HCC using only controls with liver disease.

Results from the investigation into the influence of risk factors on the association between coffee and HCC are presented as supplementary information. In summary, there was no statistically significant difference in the associations between coffee and HCC according to viral hepatitis status, smoking, BMI, T2DM, or alcohol consumption.

Caffeinated and decaffeinated coffee

Four studies reported RRs of HCC specifically for decaffeinated coffee consumption.[15, 21, 22, 32] No single study reported a statistically significant association between HCC and decaffeinated coffee consumption. Three cohort studies,[15, 21, 22] involving approximately 750,000 participants and 800 cases, reported dose-response RRs or RRs for >2 consumption categories. The pooled RR of HCC for two extra cups per day was 0.86 (95% CI 0.74-1.00; three studies). Only two studies, involving approximately 850,000 participants and 900 cases, reported RRs of HCC according to caffeinated coffee consumption in a manner suitable for dose-response analysis.[15, 22] The pooled RR of HCC for an extra two cups of caffeinated coffee was 0.73 (95% CI 0.63-0.85).

DISCUSSION

In our meta-analysis of 18 cohort studies, involving 2,272,642 participants and 2,905 cases, and 8 case-control studies, involving 1,825 cases and 4,652 controls, increasing coffee consumption by two cups per day was associated with a 35% reduction in the risk of HCC (RR 0.65; 95% CI 0.59-0.72). This is similar to previous meta-analyses.[13, 25] In a subset of studies, the association was not significantly different in participants with pre-existing CLD

at baseline, some of whom had cirrhosis. This is an important finding as the absolute risk of HCC in cirrhosis is high but may be more than halved by 5 cups/day of coffee compared to none (figure 3). The association was also not significantly different for the main exposures for HCC: high alcohol consumption, smoking, high BMI, T2DM, and HBV/HCV.[46] Data from the few studies which specified coffee type showed that increasing caffeinated and decaffeinated coffee consumption by two cups per day were associated with reductions of 27% (RR 0.73, 95% CI 0.63-0.85) and 14% (RR 0.86, 95% CI 0.74-1.00) in the risk of HCC. This is the strongest evidence to date of an association between decaffeinated coffee and HCC. It may be important for developing coffee as a lifestyle intervention in CLD, as decaffeinated coffee might be more acceptable to those who do not drink coffee or who limit their coffee consumption because of caffeine related symptoms. However, the benefits of decaffeinated coffee appear to be smaller and less certain than for caffeinated coffee.

Other major strengths of this meta-analysis are the systematic approach used to calculate a dose-response between coffee and HCC and the inclusion of a large number of participants and cases, representing a range of demographic groups (e.g. gender, nationality etc.) and the main risk factors for HCC. We did not detect effect modification by baseline CLD and HCC aetiology, although our analysis was limited by the small number of studies that provided the necessary data for these analyses.

The main limitation is that all the included studies were observational and, thus, we cannot infer causation. Observational studies are susceptible to bias and confounding, and case-control studies are at particular risk of selection and information bias. In the case-control studies, cases were mostly from hospital admissions or clinic records, which may not be representative of all HCC. Not all patients with HCC are admitted to hospital, and individual factors associated with likelihood to attend clinic and/or to participate in a research study

may be associated with coffee consumption or other risk factors (and confounders) for HCC. In addition, because of the need to interview participants, dead cases were not included.

The use of hospital controls in all except one study may also have introduced bias. Firstly, there are associations between coffee drinking and a large number of other health conditions.[47] Second, hospitals vary in the scale of their catchment areas and so hospital controls may not be representative of the populations from which cases arose especially in areas where HCC care is highly specialised.

Among the cohorts, some studies used primary liver cancer as an outcome, whereas others used HCC. All but one cohort study used cancer registries to identify cases, sometimes in combination with death records. Cancer registries are more robust for ascertainment than death records.

Residual confounding likely existed in all studies from hidden factors and misclassification of measured confounders. However, adjustment for confounders had minimal effect on the association between coffee and HCC suggesting residual effects will be small. All studies adjusted for alcohol, but several did not adjust for BMI, T2DM and HBV/HCV. Coffee was associated with alcohol in some studies, so failure to capture alcohol robustly might underestimate the inverse association between coffee and HCC.[15, 41] The cohorts generally did not adjust for HBV/HCV despite it being a major risk factor for HCC, but prevalence was likely low and we found no evidence of an effect of HBV/HCV infection on the association between coffee and HCC.

The measurement of coffee consumption may also have introduced bias in case-control studies due to recall bias. Belief that coffee was harmful may have led to overestimation of consumption in cases. However, cases may have reduced coffee consumption because liver disease slows caffeine metabolism.[48] One study used for baseline the consumption at two

years before HCC diagnosis[32] when decades before may have been more appropriate. Another study[43] reported RRs of HCC according to consumption pre- and post-identification of liver disease, the weaker pre-identification estimates were used in the meta-analysis, with minimal effect on the overall pooled RR.

In the cohorts, baseline CLD may have been present in cases given the short follow-up time of some cohorts compared to the long time for HCC to develop. However, we looked at a number of cohorts that presented data stratified by baseline CLD status and found no significant effect on the association between coffee and HCC. Setiawan et al. found that the RR of HCC for two or more cups of coffee daily compared to none remained comparable in magnitude and statistically significant when deaths in the first two years were excluded. Lai et al. found that the RR of HCC for an extra cup of coffee per day was 0.81 (95% CI 0.66-0.98) in the first ten years and 0.83 (95% CI 0.71-0.96) in the final ten years of the study. Bamia et al.[21] Hu et al.[38] and Shimazu et al.[39] reported similar findings. Thus, drinking coffee appeared to protect against HCC in participants with varying levels of undiagnosed CLD at baseline.

Our method of estimating median consumption in the reported consumption categories may have exaggerated the effect size. There was also a lack of data in most individual studies for higher levels of coffee consumption (e.g. 5 cups per day or above). As a result, we had limited ability to detect an upper threshold beyond which increasing consumption no longer provides any benefit with regard to the risk of HCC. This is evident from figure 3, which shows rapidly widening confidence intervals above four cups of coffee per day.

There was statistically significant heterogeneity between the studies; in a meta-regression analysis, it was not significantly associated with publication year, length of follow-up (cohorts only), percentage of alcohol abstainers, age or gender of participants.

Heterogeneity might be due to how consumption of coffee was measured. The included studies asked participants to estimate coffee consumption, usually by selecting from a list of predefined categories in food frequency questionnaires (FFQs). Different categories may have influenced participants' responses. There may be variation in the size of cups, preparation (e.g. boiled vs. filtered) and caffeine content; "coffee" was taken to be the pattern of use prevalent in the particular study population. Proportions of decaffeinated coffee drinkers varied markedly and were very low in certain countries (e.g. Japan and Finland).[33, 38] Higher proportions of decaffeinated coffee drinkers, such as in the United States,[22] may have attenuated the overall effect size given the weaker association found here between decaffeinated coffee and HCC.

Language bias cannot be excluded since we only included English studies, although studies found in the search were mostly in English. Generally, evidence of a significant influence in meta-analyses of language bias is weak.[49] Studies published in non-English journals may also be less rigorous and report bigger effect estimates.[50] Thus, our inclusion of English studies only is not likely to have introduced significant bias. Finally, we found evidence of publication bias using Egger's test. Adjusting for smaller unpublished studies pushed the effect size towards null but it remained statistically significant.

Our study adds to the weight of evidence considered by the IARC and WCRF that coffee is protective against HCC. However, when assessed under the GRADE criteria, the quality of evidence supporting coffee for the prevention of HCC was still "very low". This was mainly because of the lack of randomised trials, evidence of publication bias and the fact "coffee", which has various formulations with different chemical properties, is not well defined.

Mechanism of action

As discussed in detail in previous work,[18, 51] there is biological plausibility of a protective effect of coffee against HCC. The fact we found no significant effect of aetiology albeit in a subset of studies suggests that the apparent protective mechanism acts via a common pathway, such as the development of cirrhosis. Eighty to 90% of cases of HCC develop on a background of cirrhosis,[51] and several studies and a meta-analysis have reported an inverse association between coffee and cirrhosis.[18] Coffee may possess direct anti-carcinogenic properties, which is supported by our finding that the association of coffee and HCC was seen in those with pre-existing CLD, including cirrhosis. Our findings suggest a central role for caffeine, given that the association was weaker for decaffeinated coffee. Caffeine reduces HCC cell proliferation.[52] Cafestol and kahweol increase activity of phase 2 liver enzymes, which may improve metabolism and excretion of carcinogens,[53, 54] and compounds including polyphenols may ameliorate oxidative DNA damage. However, cafestol and kahweol are present only in minimal quantities in instant and filtered coffee,[55] and these varieties are popular in Japan and Finland, respectively, where studies included in this meta-analysis show inverse associations with HCC.[33, 38] Other specific mechanisms of protection might include inhibition of hepatitis virus activity[56] and prevention of T2DM.[38]

Coffee purportedly possesses a range of health effects in addition to those on the liver, including lower incidences of neurological diseases, various cancers and any-cause mortality.[47] However, randomised trials are needed of interventions to support patients at risk of HCC to increase coffee consumption before recommending an increase given the examples in other areas of where RCTs have shown observational data to be incorrect and the global scale and ubiquity of coffee consumption.[57] The potential harms of coffee also require further investigation including the reported increased risk of lung cancer and bone

fractures[47] and the deleterious effect on cholesterol, which could potentially exacerbate the already increased risk of CVD associated with certain types of liver disease.[58]

In summary, this study has shown that an extra two cups of coffee per day is associated with a one-third reduction in the RR of HCC. Our findings are significant given the increasing incidence of HCC, and the overall poor prognosis of this condition. Randomised trials should investigate the effectiveness of increasing coffee consumption in those at risk of HCC including patients with existing CLD.

COMPETING INTERESTS

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

ETHICS APPROVAL

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DATA SHARING

No additional data are available.

CONTRIBUTORSHIP

The study was conceived by all authors; The search was performed by OJK; The studies were reviewed and selected by RB and OJK; The quality of evidence assessment was performed by OJK; The risk of bias assessment was performed by JP and OJK; The data was extracted and checked by OJK and RB, respectively; The statistical analysis was performed by OJK; The manuscript was drafted by OJK and reviewed and amended by all authors. JP is guarantor.

FIGURES

Figure 1. An illustration showing how the studies included in this meta-analysis were reviewed and selected.

Figure 2. A forest plot illustrating RRs of HCC for an extra two cups of coffee per day. The RRs as reported by the individual studies are shown as squares. The sizes of the squares represent the weightings in the random-effects model. The pooled RRs (from cohorts, case-control studies and all studies) are shown as diamonds.

Figure 3. Results of a cubic spline dose-response meta-analysis of the association between coffee and HCC.

Figure 4. Filled funnel plot for the risk of HCC per extra two cups of coffee daily. Black circles represent the included studies found by our search, while white circles represent the "missing" unpublished studies detected in the trim-and-fill analysis.

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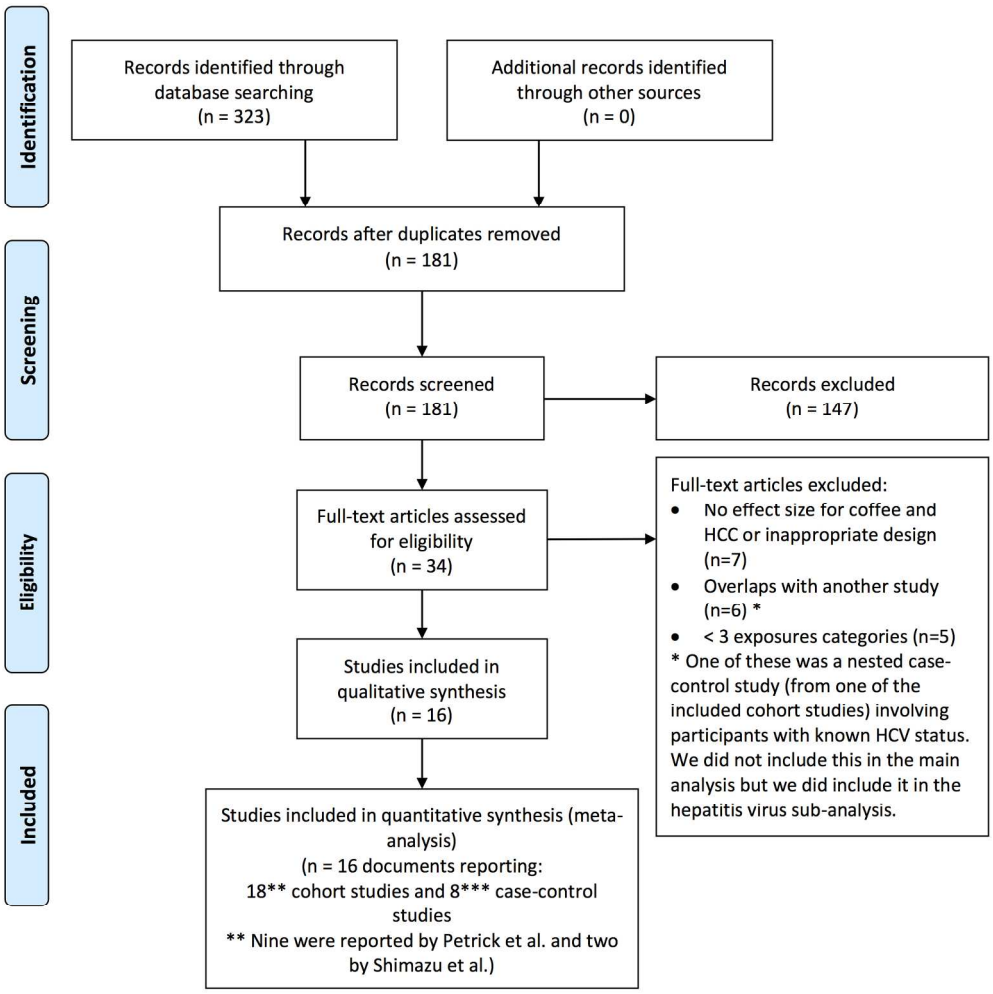

Figure 1. An illustration showing how the studies included in this meta-analysis were reviewed and selected.

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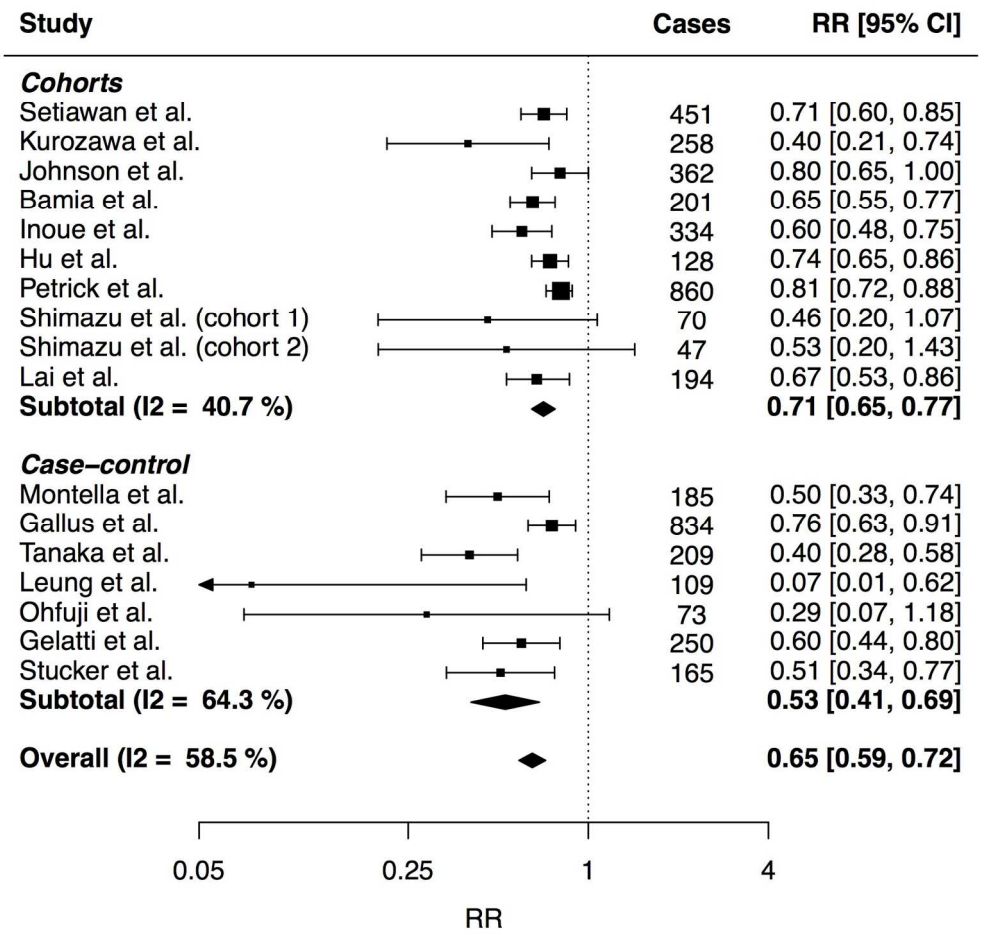

Figure 2. A forest plot illustrating RRs of HCC for an extra two cups of coffee per day. The RRs as reported by the individual studies are shown as squares. The sizes of the squares represent the weightings in the random-effects model. The pooled RRs (from cohorts, case-control studies and all studies) are shown as diamonds.

159x151mm (300 x 300 DPI)

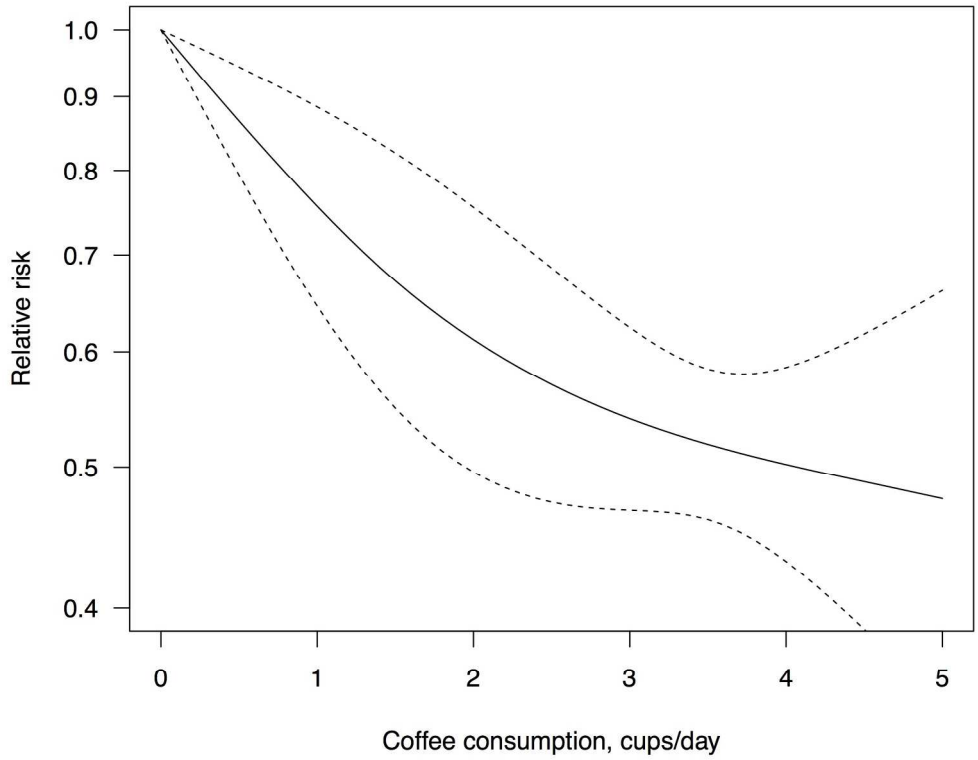

Figure 3. Results of a cubic spline dose-response meta-analysis of the association between coffee and HCC.

196x154mm (300 x 300 DPI)

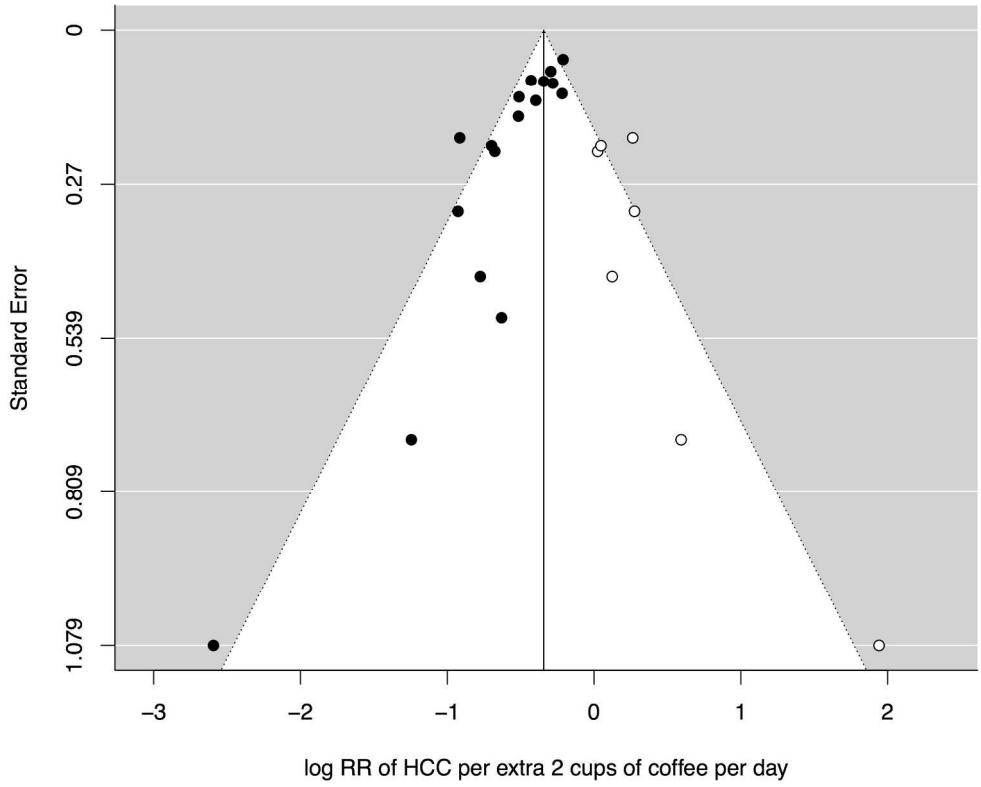

Figure 4. Filled funnel plot for the risk of HCC per extra two cups of coffee daily. Black circles represent the included studies found by our search, while white circles represent the "missing" unpublished studies detected in the trim-and-fill analysis.

221x178mm (300 x 300 DPI)

Supplementary Information: Coffee, Including Caffeinated and Decaffeinated Coffee, and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis

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THE ASSOCIATION BETWEEN COFFEE AND HCC ACCORDING TO RISK FACTOR EXPOSURE

Viral hepatitis B and C

Individual studies reported statistically significant reductions in the RR of HCC with increasing coffee consumption in participants who were HBV positive,[1] HCV positive[2] and negative for both.[3] Three studies reported RRs stratified by HBV/HCV status in a manner suitable for dose response analysis.[2-4] One of those studies reported RRs in a subgroup with HCV,[2] one in a subgroup with HBV and/or HCV[3] and one in two subgroups with (i) HBV or (ii) HCV[4] (some participants were co-infected and in both subgroups). The pooled RR of HCC for an extra two cups of coffee per day with HBV/HCV was 0.59 (95% CI 0.34-1.00; three studies) and 0.56 (95% CI 0.42-0.74; three studies) when

we included the HBV and HCV estimates, respectively, from the study with separate subgroups. Both those were weaker than the corresponding RR without HBV/HCV of 0.42 (95% CI 0.26-0.70; three studies) and the p-values for the differences were 0.50 ($\tau^2 = 0$) and 0.64 ($\tau^2 = 0$).

Diabetes and BMI

Two studies reported RRs of HCC according to coffee consumption stratified by diabetes status.[5, 6] For both studies, the RRs for an extra two cups of coffee per day were statistically significant for participants without but not with diabetes, although this may have been due to small sample size for DM. The pooled RR of HCC for an extra two cups of coffee per day was 0.79 (95% CI 0.72-0.86; two studies) without diabetes, which was larger than the corresponding RR of 0.84 (95% CI 0.69-1.04; two studies) with diabetes. The p-value for the difference was 0.70 ($\tau^2 = 0.01$).

Four studies reported RRs of HCC according to coffee consumption stratified by BMI.[5-8] The RRs for an extra two cups of coffee per day were statistically significant in two of the four studies in both the highest (above 25 and 30 kg/m2) and lowest (below 25 and 30 kg/m2) BMI categories.[6, 8] For the other studies, the RRs were statistically significant in the highest BMI category only (above 25 kg/m2 for both).[5, 7] In all four studies, the associations were stronger in the highest BMI category than the lowest. The pooled RR for an extra two cups of coffee per day was 0.72 (CI 95% 0.63-0.81; four studies) in the highest BMI category, which was larger than the corresponding RR in the lowest of 0.78 (95% CI 0.71-0.86; four studies). The p-value for the difference was 0.13 ($\tau^2 = 0$).

Alcohol consumption

Five studies reported RRs of HCC according to coffee consumption stratified by alcohol intake in a manner suitable for dose-response analysis.[3-5, 8, 9] The RRs of HCC for an

extra two cups of coffee per day were statistically significant in three studies for the highest categories of alcohol consumption[3, 4, 8] and in three studies for the lowest.[5, 8, 9] The pooled RR of HCC for an extra two cups of coffee per day in the highest category of alcohol consumption was 0.63 (95% CI 0.51-0.77; five studies) compared to 0.71 (95% CI 0.63-0.79; five studies) in the lowest. The p-value for the difference was 0.53 ($\tau^2 = 0$).

Smoking

Five studies reported RRs of HCC for three or more categories of coffee consumption separately for smokers and non-smokers[4-6, 8, 9]. The non-smoker groups mostly contained never smoker and ex-smokers. The RRs of HCC for an extra two cups of coffee per day were statistically significant in four studies for smokers [4-6, 8] and in two studies for non-smokers [5, 8]. The pooled RRs were 0.68 (95% CI 0.55-0.83; five studies) for smokers and 0.78 (95% CI 0.70-0.87; five studies) for non-smokers. The p-value for the difference was 0.13 ($\tau^2 = 0$).

PRISMA-P PROTOCOL

Section and topic	Checklist item
ADMINISTRATIVE INFORMATION	
Identification	We will perform a systematic review with meta-analysis of the relationship between caffeinated and decaffeinated coffee and hepatocellular carcinoma (HCC). There are existing meta-analyses on coffee and HCC but none on decaffeinated coffee or the influence of HCC aetiology.
Registration	Our protocol is unregistered
Authors	Oliver John Kennedy[1]; Paul Roderick[1], Ryan Buchanan[1], Jonathan Fallowfield[2], Peter Hayes[2], Julie Parkes[1] 1. Primary Care & Population Sciences Faculty of Medicine University of Southampton 2. MRC Centre for Inflammation Research University of Edinburgh
Support	There are no sponsors or financial interests to declare.
INTRODUCTION	
Rationale	Primary liver cancer is the sixth most commonly diagnosed cancer worldwide. Hepatocellular carcinoma (HCC) is the most common subtype of primary liver cancer. Of concern is the

	global increase of non-alcoholic steatohepatitis/metabolic syndrome which can progress to HCC in the absence of cirrhosis. A number of studies have shown drinking coffee is inversely associated with the risk of diseases affecting the liver, including HCC.
Objectives	To determine quantitatively the relationship between caffeinated and decaffeinated coffee and the risk of HCC. To investigate whether the relationship between coffee and HCC is influenced by pre-existing liver disease or specific risk factors (e.g. EtOH, HBV/HCV, metabolic factors)
METHODS	
Eligibility criteria	We will include studies in our meta-analysis that: • are cohort or case-control studies • report effect sizes (RRs, OR, HRs) for primary liver cancer/HCC according to coffee intake (adults only). We will exclude studies that: • report no dose-response or provide insufficient information for one to be computed. • are published in a language other than English.
Information sources	Searches will be performed for published studies using Web of Science, Pubmed and Embase, and no limitation of date of publication will be imposed. Manual searches of reference lists will be performed.
Study selection process	Duplicates will be removed before two authors screen studies, first by abstracts and titles followed by full text.
Data collection process	The following data will be extracted from the included studies: • first author, date of publication, country, the design of the study, the exclusion and inclusion criteria, the estimates and adjustments, the numbers of participants (or controls) and cases, the methods of measuring exposure and case identification, cohort follow-up (time, losses), whether baseline liver disease was excluded. • the most rigorously adjusted effect sizes and effect sizes stratified by pre-existing chronic liver disease, alcohol consumption, BMI, hepatitis B and C virus status, diabetes, and type of coffee. • RRs for total caffeinated and decaffeinated coffee consumption, including RRs stratified by pre-existing liver disease and aetiology.
Data items	We will assume that hazard ratios, odds ratios and relative risks are the same.
Outcomes and prioritization	HCC stratified by risk factors / aetiology / type of coffee
Risk of bias	Newcastle-Ottawa scale shall be used for risk of bias assessment
Data synthesis	We will calculate RRs for a two cups/day increase and for 1-5 cups per day, where possible stratified by risk factors. I2 and Cochrane Q will be used to assess heterogeneity. We will re-run the analysis while omitting each study.
Additional analyses	Eggers test and a trim-and-fill analysis will be used to

	investigate publication bias.
Confidence in cumulative evidence	GRADE will be used for assessment of evidence quality

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6. Petrick JL, Freedman ND, Graubard BI, et al. Coffee Consumption and Risk of Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma by Sex: The Liver Cancer Pooling Project. Cancer Epidemiology Biomarkers & Prevention 2015;**24**(9):1398-406 doi: 10.1158/1055-9965.epi-15-0137.
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Research Checklist: Caffeinated and Decaffeinated Coffee Consumption and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis

MOOSE CHECKLIST FOR META-ANALYSES OF OBSERVATIONAL STUDIES.

	Page/table/figure	Details
Reporting of background should include		
Problem definition	4	Primary liver cancer is the sixth most commonly diagnosed cancer. HCC is the most common subtype of primary liver cancer. HCC usually develops in people with cirrhosis but HCC without cirrhosis is becoming more common because of the increasing prevalence of non-alcoholic steatohepatitis/metabolic syndrome.
Hypothesis statement	4	Coffee has been associated with a reduced risk of hepatocellular carcinoma (HCC). It is unclear whether the inverse association also exists for decaffeinated coffee or whether it is influenced by HCC aetiology.
Description of study outcomes	5	HCC (all causes)
Type of exposure or intervention used	5	Caffeinated and decaffeinated coffee consumption
Type of study designs used	5	Observational studies
Study population	5	All populations.
Reporting of search strategy should include		
Qualifications of searchers (eg librarians and investigators)	5	The authors did the searches
Search strategy, including time period used in the synthesis and key words	5	Search term: (“odds” OR “risk” OR “hazard” OR "OR" OR "RR" OR "HR") AND “coffee” AND (“liver” OR “hepatocellular*) AND (“cancer“ OR “carcino*” OR neoplas*). We placed no restrictions on publication dates.
Effort to include all available studies, including contact with authors	5	All studies eligible were included.
Databases and registries searched	5	Web of Science, Embase, and PubMed
Search software used, name and version, including special features used (eg explosion)	5	Ovid was used for searching Embase.
Use of hand searching	5	References of pertinent studies were

(eg reference lists of obtained articles)		searched manually.
List of citations located and those excluded, including justification	8, Figure 1	Figure 1 illustrates the process for selecting the studies for inclusion in this meta-analysis. We will provide citations for the excluded study by request.
Method of addressing articles published in languages other than English	5	English studies only.
Method of handling abstracts and unpublished studies	5	Only published studies (abstracts were not excluded).
Description of any contact with authors	5,7	Published data only.
Reporting of methods should include		
Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	5,6	Detailed exclusion and inclusion criteria are specified.
Rationale for the selection and coding of data (eg sound clinical principles or convenience)	6	The data extracted from each study related to study type, categories of exposure, outcome, adjustment for confounding and population characteristics.
Documentation of how data were classified and coded (eg multiple raters, blinding and interrater reliability)	5,6	One author extracted the data which was then checked for accuracy by a second author.
Assessment of confounding (eg comparability of cases and controls in studies where appropriate)	6	The risk of bias was investigated by the Newcastle-Ottawa scale. The direction and magnitude of overall adjustment was calculated.
Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	6	The risk of bias was investigated by two authors separately and the conclusions discussed for agreement.
Assessment of heterogeneity	6,7	Heterogeneity was assessed using I^2 and Cochran's Q.
Description of statistical methods (eg complete description of fixed or random effects models, justification of whether	7,8	We used a random effects meta-analysis after first calculating a RR of HCC for an increase in consumption of 2 cups/day for each study.

the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated		
Provision of appropriate tables and graphics	Figure 1	Figure 1 shows the search and study selection process.
Reporting of results should include		
Graphic summarizing individual study estimates and overall estimate	Figure 2	
Table giving descriptive information for each study included	9,13, Tables 1 and 2	
Results of sensitivity testing (eg subgroup analysis)	14,15	We report results of several sensitivity analyses investigating the effect of study type and study quality.
Indication of statistical uncertainty of findings	13	95% confidence intervals are provided / p-values are provided where necessary.
Reporting of discussion should include		
Quantitative assessment of bias (eg publication bias)	13	We used Egger's test and a trim-and-fill analysis.
Justification for exclusion (eg exclusion of non-English language citations)	8, Figure 1	Studies were excluded if they did not provide a dose-response estimate or allow one to be calculated.
Assessment of quality of included studies	8, Table 1	We test if the effect size varies when we restrict the analysis to high quality studies.
Reporting of conclusions should include		
Consideration of alternative explanations for observed results	16-19	We discuss residual confounding and possible sources of bias.
Generalization of the conclusions (eg appropriate for the data presented and within the domain of the literature review)	16	The vast majority of the cohort studies were of the general population.
Guidelines for future research	21	Last paragraph in the main text.
Disclosure of funding source	21	See " FUNDING" section.